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SEPTEMBER 1927

Polygonum: Sectio Tovara - - - - - - - Theo. Holm 1

Sexual Dimorphism in Mucorales. I. Intraspecific Reactions
A. F. Blakeslee, J. L. Cartledge, D. S. Welch, and A. D. Bergner 27

Sexual Dimorphism in Mucorales. II. Interspecific Reactions
A. F. Blakeslee and J. L. Cartledge 51

The Nature of Chromosomes. I. Effects of Reagents on Root Tip Sections of Vicia faba. Contributions from the Hull Botanical Laboratory 372
R. O. Earl 58

Variation in Sugar Content in Potato Tubers Caused by Wounding and Its Possible Relation to Respiration - - E. F. Hopkins 75

Abnormal Sex Organs of Mnium medium - - George S. Bryan 89

Briefer Articles

A Watering System for Culture Vessels - - - Alfred Aslander 102

Nomenclatorial Note on Smithiantha fulgida (Ortg.) Cheney, comb. nov.
Ralph H. Cheney 105

Current Literature

Book Reviews - - - - - - - - - - - - - 106

Notes for Students - - - - - - - - - - - - - 110

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Volume LXXXIV Number 1

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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Issued September 30, 1927

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September and October 1927

Humanizing the Sciences

Comment on *The Nature of the World and of Man* by sixteen members of the Faculty of the University of Chicago is running high.

Whatever their intentions toward college Freshmen, which are of no very great interest to the world outside the universities, the sixteen scientists have written sixteen fireside lectures ranging from astronomy to psychology, which the critics rank as the best of recent attempts to humanize the sciences. Opinion runs as follows:

"One of the finest and most engrossing popularizations of current science in existence. . . . . It is simple, clear, concrete, reliable. One emerges from it the richer by solid information and definite concepts."—HENRY HAZLITT, *New York Sun.*

"A manual of the physical and biological sciences of which it would be hard to speak too highly. The volume is so many things that an outline ought to be but frequently is not. It is selective in the sense of plucking the real heart out of a body of data."—*New York Times Book Review.*

"It is unexcelled for the general reader and many of those who have had scientific training will here learn for the first time what it is all about. Above all, it is vigorously inspiring."—*New York Herald-Tribune.*

"For the story is well told, well illustrated, and well colored with human significance. Certainly the volume is accurate; the names of the various contributors guarantee that. Certainly it is interesting and readable, and popular without being diluted."—*The Nation.*

"It sweeps on from one great truth to another in a way that gives one a profound respect for the recent progress of science."—*Saturday Review of Literature.*

"It is fascinating reading for anyone who has a spark of that divine curiosity about the ways of life which causes man to seek, and seeking, to progress."—*Chicago Tribune.*

That the orientation course upon which *The Nature of the World and of Man* is based is helping the students build a workable philosophy of life is indicated in a large body of comment from these students themselves. The following statements are representative of the student attitude toward the course:

"To me this course has been most stimulating. It has given a new and broader conception of the universe. It has aroused in me a new interest and spirit of curiosity. I feel that to some extent, I have learned to value the scientific spirit. The influence that science has upon our lives is better appreciated. I believe that the course was intended to present a brief but comprehensive picture of everything that concerns us, to shake up our minds, and to stimulate us to a little thought. It has done those things for me. I am eager to take more science courses and find out how and why the universe is as it is."

"I find the spirit of science to be open-minded, strong, fearless, and wholesome. It is strange to consider one's self related to the great universe of things. I am very enthusiastic about the course. The realization or the apprehension of the infinitude of the heavens, the universe of the atom has put me in my proper place in the cosmos."

"This course is called an orientation course. This expresses well its aim and what it has

done for me. It has taken me out of the universe and has set me at a point outside where I could see everything that was going on and look at the ways of the universe from an impartial perspective. It has given an introduction to the natural sciences and their interrelationship. The greatest benefit I have received, a benefit which sinks all other results into insignificance, is that it has caused me to ask myself such questions as 'Who am I?' 'What am I?' and 'Where am I?' It has changed my outlook on the world."

Two printings of *The Nature of the World and of Man* have already been exhausted and a new printing of 15,000 has been ordered. This will include a revision of the chapter on "The Nature of Chemical Processes" by Julius Stieglitz. As re-written it will simplify the subject of chemistry for the layman. *THE NATURE OF THE WORLD AND OF MAN.* By SIXTEEN MEMBERS OF THE FACULTY OF THE UNIVERSITY OF CHICAGO. *$4.00, postpaid $4.15.*

* * *

Gangs

Gang activities are familiar enough in cosmopolitan cities, but strangely, there has been no adequate sociological study of them. The forces that explain gangdom, adult and juvenile, have been brought to light by Professor Frederic M. Thrasher in *The Gang*, a book which critics believe destined to become the standard source book on the subject.

Professor Thrasher spent six years in his study of gangland—the poverty belt which surrounds Chicago's loop district. He lived with the gangsters, interviewed their leaders, and became intimately acquainted with gang life from the inside. Mingling with the "Murderers," the "Deadshots," and members of hundreds of other gangs, he learned their mysteries and secret signs, their attitudes toward society, and toward each other. And he found in investigating 1,313 gangs of all ages and sizes, the facts which explain the sensational features of gang life which are flaunted in the headlines.

He looks upon the gang as a social makeshift, which naturally appears in the cracks of the community and inevitably fosters the spread of crime. Abandoning the idea of a gang instinct, he attempts through a careful study of the natural history, structure, and functioning of the gang, to arrive at the true causes for its existence.

Mr. Thrasher has collected a vast amount of intensely interesting material which he discusses with a minimum of seminar jargon. A large part of the case material was secured from the gang boys themselves. A typical case is the following, obtained in an interview with a gang boy:

BILLY, THE BRAINS

Billy was the brains of the gang. He was "educated," a high-school boy too. He would work sometimes, but not often. The kids would bring their "stuff" to him. One day we had a big fight over it when we were robbing a merchandise car; we had cigarettes, pop, and a lot of other stuff. Billy had his stuff put away in a box with straw on top of it. The watchman looked at the straw, but Billy told him it was for a rabbit. Billy would sell "cartoons" (of cigarettes) for a half a dollar apiece.

Billy would plan things for our gang. He would get us a place to sleep when we were bumming away from home. He would get us keys to the bread boxes, so that we could get food when we were hungry. We would get the bread after the bakers left it early in the morning before the stores opened. . . . . Billy would find us a place to sleep in some house or basement. He would go around everywhere to see if there was a place to sleep or rob; he was a regular investigator.

Henry Zorbaugh of New York University comments: "Dr. Thrasher's monograph should be of interest to the sociologist and psychologist as a model of social research and a contribution to our knowledge of human behavior; to educator and social worker as an illuminating background against which to consider the practical problems of boy adjustment; and to the romantically minded as a fascinating story of 'the web of life' as it is spun in a great city."

Harry Hansen says in the *New York World:* "Here is a remarkable document and one that ought to be a source book for years to

come. . . . . He has dug right down into boyland, gone to the root of the problem, and produced a book that will become an authority." *THE GANG*. By FREDERIC M. THRASHER. *$3.00, postpaid $3.15.*

* * *

Ernest DeWitt Burton

During a half-century of research Ernest DeWitt Burton achieved an international reputation as a New Testament scholar. In two years of extraordinary activity as President of the University of Chicago, he became equally well known as an educational executive. In addition, a leader in missionary and denominational enterprises, he offers an inspiring example of life lived to the full.

Dr. Burton's personality made a deep and lasting impression on those who knew him. "In him," says Henry Justin Smith, "were concentrated the mental vigor of youth and the benignity of age; the devotion of a missionary and the acuteness of a worldling; the simplicity of a plain man and the delicacy of a true aristocrat."

Of his associates, no one is better qualified to portray this great and enormously attractive individual than Thomas W Goodspeed, who has been an intimate witness of his life-activity. In *Ernest DeWitt Burton: A Biography*, Mr. Goodspeed has followed his career from his student days at Denison, through his work as head of the Oriental Educational Commission, chairman of the China Educational Commission, and President of the University of Chicago, to his last days.

Mr. Goodspeed has not attempted to idealize Ernest DeWitt Burton, but to present him as he was in every period of his life. *ERNEST DeWITT BURTON*. By THOMAS W. GOODSPEED. *$3.00, postpaid $3.10.*

*

Dr. Burton left unpublished some exceedingly valuable work, and this has been preserved in two posthumous volumes, *Christianity in the Modern World*, and *A Short Introduction to the Gospels*.

The first of these volumes brings out one of the most attractive phases of Dr. Burton's character. Internationally known as a New Testament scholar, and as President of the University of Chicago, Ernest DeWitt Burton has another side no less great, but not as well known. This was Dr. Burton the Christian and exemplar of the practical Christian life, the man of deep religious experience and conviction.

This phase of his character is uppermost in this collection of his fugitive utterances on religious themes. Here are his great *apologia pro vita sua*, "Why I am content to be a Christian"; his views upon Jesus and Paul; and the essence of his thinking on Christian education and world-problems.

This book is fittingly concluded with "Christian Education in China," Dr. Burton's last public address.

The second of the two volumes incorporates his final conclusions in a work representing one of his most important projects. Dr. Burton continued during his last years the researches concerning the origins of the Four Gospels which had been the basis of his valued *Short Introductions to the Gospels*. He made further investigations regarding the dates of the Synoptic Gospels, and approached the Fourth Gospel from a new point of view, applying the method of source criticism which he had already used with conspicuous success in the case of the Synoptic Gospels.

These significant advances have been incorporated by Harold R. Willoughby in a new revision of the volume, which brings to biblical students valuable new material. Two important sections of fresh material found in Dr. Burton's files have been added. The first renders an exceptional judgment regarding the dates of the Synoptic Gospels. The second outlines a unique theory concerning the composition of the Fourth Gospel which illuminates the Johannine depiction of Jesus.

This authoritative revision places before students of the Gospels those facts concerning the purpose and point of view of each of them which are most necessary for an intelligent understanding of them. *CHRISTIANITY IN THE MODERN WORLD*. By ERNEST D. BURTON. *$2.00, postpaid $2.10. A SHORT INTRODUCTION TO THE GOSPELS*. By ERNEST D. BURTON AND HAROLD R. WILLOUGHBY. *$1.75, postpaid $1.85.*

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The "University of Chicago Junior College Series" of French texts, initiated not long ago with Otto F. Bond's *The Sounds of French* and *An Introduction to the Study of French*, has, because of the departure of its methods from those generally in use, become a center of controversy. Mr. Bond maintains that most of his critics have mistaken his aims. Answering one of them, Colley F. Sparkman, in the *Modern Language Journal*, he says: "I am afraid that Mr. Sparkman suspects me of having wished upon the public a substitute for a composition grammar or some *bête difforme* lacking synthetic legs and a *raison d'être*, since he would have me add various types of exercises involving composition. Let me point out that the Preface (page viii) states the purpose of the book as being to enable the student to read (not write or converse!) in a short time and to prepare him for better and more rapid progress in acquiring expression power.

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* * *

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COWLES

So extensive a program as this book presents has seldom been attempted, much less accomplished so successfully. It is an outline of our knowledge of the physical and biological world and man's relative position in it: a bird's-eye view of the universe in the light of modern science.

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VOLUME LXXXIV NUMBER 1

THE
BOTANICAL GAZETTE

September 1927

POLYGONUM: SECTIO TOVARA

THEO. HOLM

(WITH PLATES I, II)

It is often interesting to follow the history of some genus from the earliest works on botany until recent time. So far as concerns *Polygonum*, several species were known as far back as the time of DIOSCORIDES, and it is really remarkable to see how some of the oldest names have been preserved, being applied to sections or subgenera. The earliest attempts to define the species depended upon the mere habit of these plants, and sometimes their properties. The name *Polygonon* was used by DIOSCORIDES for *Polygonum maritimum* and *P. persicaria* var. *incanum; Hydropiper* for *P. hydropiper;* and *Helxine* for *P. dumetorum*. Our *Polygonum convolvulus* was called *P. hederaceum* by FABIUS COLUMNA, while DODONAEUS and LOBELIUS had it under the name *Helxine cissampelos*. *P. bistorta* is BRUNFELS' *bistorta; P. aviculare* was called *Centumnodia* by BRUNFELS, *Sanguinaria* by LOBELIUS "a cohibendo sanguine," and this is the only species of the genus which JOHANNES BAUHIN (3) has enumerated as being named for a saint, "S. Innocentii herba." The name *Polygonum*, however, was also used by the early writers for several other plants of the same habit, for instance *Radiola*, *Lepigonum*, *Herniaria*, etc., according to CASPAR BAUHIN (2). While the genus had thus been elaborated to some extent by writers before BAUHIN, this author deserves credit for being the first to present a systematic arrangement of the species with generic names: *Bistorta*, *Persicaria*, *Hydropiper*, *Polygonum*, and *Convolvulus* ("minor semine

triangulo"). He was the first writer to use binomials, and his work, containing a large number of synonyms, dating back to the earliest writers, represents the most important contribution to scientific botany of that period.

The arrangement of the genera by BAUHIN was of course anything but natural from a modern point of view. The habit of the plants and sometimes their properties were considered the most important. Consequently the genera described by BAUHIN are frequently widely separated, as may be seen from *Polygonum*. *Hydropiper*, for instance, is placed among acrid herbs (2) such as Cruciferae and Piperaceae. *Bistorta* is placed near *Statice* and *Aroideae*, evidently on account of the large tuberous rhizomes. *Polygonum*, our *P. aviculare*, heads a group of plants among which are *Sedum*, *Sempervivum*, and *Saxifraga*, several of which have small crowded leaves. Finally his *Convolvulus*, which comprises the true genus and our *P. convolvulus*, is in the same chapter as *Smilax*, *Bryonia*, *Lupulus*, and *Vitis*. An alphabetical arrangement of the genera might have been more appropriate, but BAUHIN preferred to arrange the plants in accordance with his idea of natural affinities, and his system is indeed far superior to those proposed by his predecessors. In the course of the century that elapsed between the works of BAUHIN and TOURNEFORT (24) several new species of *Polygonum* were described, notably in the writings of PARKINSON (1640), MORRISON (1680), RAY (1686), and PLUKENET (1696), and among these species was our *Polygonum virginianum*: "*Persicaria* frutescens maculosa, Virginiana, flore albo" Parkinson, subsequently described in the same manner by RAY. Contrary to BAUHIN, who named only the genera but described the species, TOURNEFORT described the genera, enumerating the species with the diagnoses given by the respective authors, without appending any diagnoses himself. His system was based upon the floral structure, and carried through with remarkable care. The plants were arranged in classes, with sections comprising the genera and species, accompanied by numerous figures. His classification of the genera, however, was in many cases anything but natural. TOURNEFORT never made any use of the works of RAY, who drew the distinction between Cryptogames and Phanerogames, and who established the two groups Monocotyledones and Dicotyledones. The the-

ory proposed by CAMERARIUS was not accepted by TOURNEFORT. Nevertheless some genera, of which the floral structure is very uniform, were arranged in the same section, as for instance the *Polygonum* alliance. This is described in the 15th class, "flore apetalo seu stamineo," together with *Rumex*, several Chenopodiaceae, besides *Asarum*, *Herniaria*, *Paronychia*, *Alchemilla*, and some others. At the end of the same class we find the Gramineae, Cyperaceae, *Ricinus*, *Hippuris*, and some Urticaceae. TOURNEFORT described *Persicaria*, *Polygonum*, *Fagopyrum*, and *Bistorta* as genera, but he referred *Helxine* (*Polygonum convolvulus*) to *Fagopyrum*, together with *F. scandens americanum* (Hermannus), known now as *Polygonum scandens*. The floral structure being so very uniform, TOURNEFORT inserted also some characters taken from the habit, for instance: "flores in foliorum alis nasci" (*Polygonum*); "spica" and "radices tuberosae" (*Bistorta*); "occurrunt etiam species quibus praeter flores et semina innascuntur tubercula" (*Bistorta alpina minor* Bauhin), which is our *P. viviparum*. It would be very difficult, however, to determine the species of his *Persicaria; P. virginianum*, for instance, appears only with the brief diagnosis of PARKINSON, and may hardly be distinguished from the numerous other Persicariae.

These various systems were intended for demonstrating the natural relationship of the plants by means of some arrangement of the genera. From a practical viewpoint, however, the systems were a failure, since they did not facilitate the determination of the plants, and especially not the genera. Some other kind of system was needed, one that would make the knowledge of plants more accessible. The Linnaean system, artificial as it was, facilitated the determination of plants, genera as well as species. The method to be followed when using this system is so simple that it was illustrated by only 24 small figures on one plate (Genera plantarum, ed. 2, Leyden 1742). The only difficulty in using this system is the fact that there are several genera of which the number of stamens is not the same in all the species of the same genus. In *Polygonum*, for instance, a member of the eight class, all the species are placed together, even though the number of stamens fluctuates between five and eight. This is pointed out by LINNAEUS himself, where under *Persicaria* the statement is made: "Stamina et pistillum quoad numerum in hoc genere, incerta

sunt." In his earlier years LINNAEUS (13) regarded *Persicaria*, *Bistorta*, *Polygonum*, and *Helxine* as genera. There was only one species of *Polygonum*, *P. aviculare*; *Helxine* comprised *Polygonum convolvulus* and its allies, besides *P. arifolium* and *P. sagittatum*. *P. virginianum* was a member of *Persicaria*, but with the diagnosis much improved: "florum staminibus quinis, stylo duplici, corolla quadrifida inaequali." In this same work we find the earliest mention made of the nectaries, namely in *P. orientale:* "Glandulae nectariferae manifestae tot sunt, quot stamina, uti in *Helxine* videre est." The six species of *Persicaria* enumerated are so well described, with a very few words, that their identification is very easy; for instance, *P. lapathifolium:* "staminibus quinis corollae regulari aequantibus"; and *P. amphibium:* "staminibus quinis corollae superantibus," etc. These genera were later referred to *Polygonum*, but the species, 27 in all, were arranged in the same manner as before under the respective sections *Bistorta*, *Persicaria*, etc. In describing *P. scandens* LINNAEUS mentions a pore at the base of the petiole, a structure which seems to be very rare in the genus, and described only as characteristic of *P. cilinode* Michx. in the monograph by MEISNER (14). Thus, while LINNAEUS adopted the classification of *P. virginianum* as proposed by TOURNEFORT and his predecessors, namely, as a *Persicaria*, ADANSON (1) established the genus *Tovara*, based upon this species.

ADANSON named the family Persicariae, containing the genera *Tovara*, *Persicaria* Tour., *Polygonum* Tour., *Fagopyrum* Tour., *Helxine* L., and *Bistorta* Tour. The generic characters of *Tovara* were given as follows: "épilâche terminale, calice 4 divisions, étamines 5, stigmates 2 cylindriques, graine lenticulaire." With reference to the nectaries, this author observed them in species of *Persicaria* and *Fagopyrum*. With the exception of the inflorescence, a loose flowered spike, the other characters are about the same as those LINNAEUS mentioned in his diagnosis. ADANSON, however, did not notice that the lobes of the perianth are of unequal length, as correctly described by LINNAEUS. The very little that was actually known about *Tovara*, and evidently the lack of complete material, especially of the fruiting stage, must have been the reason why it became suppressed for so many years. Even MEISNER would not recognize *Tovara*, even as a mere section. The genus *Polygonum* L. was by this author di-

vided into seven sections: *Bistorta*, *Fagopyrum*, *Persicaria*, *Avicularia* (sectio *Polygonum* L. partim), *Tiniaria* (sectio *Helxine* L. partim), *Aconogonon* Meisn., and *Amblygonon* Meisn. Our *Polygonum virginianum* appears here as a member of *Persicaria*, and very little of importance is added to the diagnosis as given by LINNAEUS and ADANSON, while the figures on plate III and the explanation of these contain several new points: the diagram of the flower, the nectaries, the pistil with the two long styles slightly diverging at the apex and reflexed at maturity of the fruit, all being characters of importance, and sufficient for accepting *Tovara* as a section; and these figures were drawn by MEISNER himself.

In the works of TORREY (**23**) and GRAY (**8**) we find *Tovara* described as a monotypic section, and TORREY has given an excellent diagnosis comprising the most important sectional characteristics, among which may be cited the very long virgate spike, with one or two flowers from each bract; the unequally four-parted calyx (perianth); the five somewhat unequal stamens; the rigid, parallel styles exserted when in fruit, bent obliquely downward at the base so as to form an obtuse angle with the ovary, the summits recurved; stigmas minute; achenium large, strongly curved on both sides, beaked with the persistent styles; embryo curved against one of the edges of the albumen, the cotyledons oblong. TORREY, however, does not mention the nectaries within this or in any of the other sections; the habit is given as perennial only. The description given of *Tovara* in GRAY'S manuals agrees with the one by TORREY, and even in the last edition (1908) the nectaries have been overlooked, and no additional statement is made as to the vegetative reproduction.

In BENTHAM and HOOKER'S *Genera plantarum* (1883) *Tovara* is described as a section, with *Antenoron* Rafin. as a synonym. Two species are said to be known, one in North America, another in Japan, but closely related to each other. Ten sections are recognized by BENTHAM and HOOKER, among which *Avicularia*, *Persicaria*, *Cephalophilon* (*P. sagittatum*, *P. arifolium*), *Tiniaria*, *Pseudopolygonella*, and *Tovara* are represented in North America. Furthermore, DAMMER (**7**) accepts *Tovara* as a section, but gives only a rather incomplete diagnosis as compared with the one by TORREY. According to DAMMER the Japanese species is *Polygonum filiforme* Thunbg., which

by MEISNER is not placed very close to *P. virginianum*, nor does the brief diagnosis indicate any close relationship. THUNBERG (22), however, gives a more comprehensive description of the species (*P. filiforme*), mentioning the four-parted perianth, the five stamens, the two styles being as long as the filaments of the stamens. The spikes are said to be filiform, and the leaves ovate with the ochreae ciliate, characters that might indicate identity with *Tovara*, at least at the stage of flowering, but the important fruiting stage is not included in the diagnosis. ADANSON mentions that a species of *Tovara* grows in China, from which a blue dye is obtained; this species, however, may hardly be *P. filiforme*, since THUNBERG only mentions *P. chinense* L., *P. barbatum* L., and *P. aviculare* L. as being frequently cultivated for that purpose.

Finally, BRITTON and BROWN (4) have not referred other species than *P. aviculare* and its nearest allies to *Polygonum* as a genus, raising the sections *Tovara*, *Persicaria*, *Bistorta*, *Tracaulon* (*P. sagittatum*), and *Tiniaria* to generic rank. Of these *Tovara* is described as annual or perennial herbaceous; the two styles are said to be recurved or curled, and the figure shows the fruit with the styles diverging from the base and reflexed, errors that would have been avoided if the authors had examined the living plant and compared the diagnosis given by TORREY. The floral nectaries are not mentioned by these authors, and the characteristic extra-floral nectary in *Polygonum cilinode* and *P. scandens* has also been overlooked.

The classification adopted by BENTHAM and HOOKER, keeping *Polygonum* intact but distinct from *Fagopyrum*, appears the most natural. It is a polymorphous genus as to habit and floral structure, but the sectional distinctions are not sufficient for the establishment of separate genera, as has been done by BRITTON and BROWN. For instance, the four-parted perianth, the number of stamens fluctuating between four and six, and the two styles are not characteristic of *Tovara* alone, for these structures recur in *Tephis* Adans., a section closely related to *Avicularia*, and consequently with a habit widely different from *Tovara*. A synopsis of the sections, showing these various structures, is given by MEISNER, but, as already stated, this author did not recognize *Tovara* as a section, placing the species as a

member of *Persicaria*. Moreover, the name *Helxine* is changed to *Tiniaria*, which according to SPRENGEL (21) was proposed by MARCEL DE BORDEAUX for *Polygonum convolvulus*.

We have thus in the genus *Polygonum* an example of very old systematic botany, where a genus has been preserved, and where some of the species have been understood in a very natural manner, corresponding with our modern viewpoint. *Polygonon* of DIOSCORIDES comprises the sections *Avicularia* and *Persicaria;* his *Helxine* corresponds with the section of the same name by LINNAEUS, but afterwards changed to *Tiniaria;* the *Bistorta* of BRUNFELS is our subsection *Bistorta*. It is also interesting to see that FABIUS COLUMNA regarded *Helxine* as a *Polygonum*, calling it *P. hederaceum*, a case of generic synonymy. According to LINNAEUS all these were genera, but only for some time, his final classification reducing them to one genus, "*Polygonum*," with sections. With the exception of *Fagopyrum*, which is not included in *Polygonum* by BENTHAM and HOOKER, the other species are still regarded as members of the genus; however, with some modifications of the sections. For instance, *P. sagittatum* and *P. arifolium* are removed from *Tiniaria* and referred to *Cephalophilon; Bistorta* is removed to *Persicaria; Tovara* is established, etc.

While the more recent classification of the species of *Polygonum* is based principally upon the floral structure, the general habit has also been considered, but to a much less extent. From this particular viewpoint the genus is not very interesting, judging from the relatively scant information obtained from the literature, and the manuals seldom contain other statements than "annual or perennial." "Ours all herbaceous with fibrous roots except in *P. viviparum*" is the generic characterization as given in the seventh edition of GRAY'S manual, and "a bulb-like caudex" is attributed to the section *Bistorta*. The subterranean stem structure of some of these has been described carefully and explained many years ago; for instance, *Polygonum amphibium* (10), *P. bistorta*, and *P. viviparum* (17), and ought to have been considered. With reference to the section *Tovara*, *Polygonum virginianum* is not well known from a morphological point of view, and the following notes may serve as a contribution to the knowledge of this rather singular species. Being an inhabitant of

shady woods, I have for the sake of comparison included the anatomical structure of some species of the sections *Persicaria*, *Avicularia*, and *Cephalophilon*, representing heliophilous types.

SUBTERRANEAN STEM.—MEISNER has figured the seedling stage. The cotyledons are epigeic, elliptic; there is a distinct hypocotyl, and the primary root is relatively long, slender, and ramified. I have not seen the stages intermediate between the seedling and the mature plant (fig. 1), but judging from the structure of the subterranean stem, which in several respects agrees with that of *Polygonum amphibium* (10), it seems probable that the first stolons are developed from cotyledonary buds common to several species of the genus. Fig. 1 shows a horizontally creeping, subterranean stem terminated by an erect floral shoot (*St*). Four stolons are developed, densely covered with tubular bladeless leaves, but with no roots. Secondary roots, on the other hand, are well represented upon the old stem internodes; they are thin, amply ramified, and proceed from the nodes or between these. The old stem is two years old, and considerably thicker than the stolons, which develop into aerial (mostly floral) shoots in their second year of growth. The mother stem does not persist, and withers gradually. The vegetative reproduction thus enables the plant to become distributed and to form new individuals. "Perennial by stolons" would be the proper characterization of this species, instead of simply "perennial," and I have never found any specimens that were annual.

FLOWER (figs. 2–5).—All the flowers are lateral, arranged in a long, thin, spicate inflorescence, and the subtending leaves are merely represented by ochreae, very remote from one another. Each of these ochreae subtends a three-flowered, secondary inflorescence of the rhipidium type, where the apparently central flower is the youngest.

As regards the diagram of the flower (fig. 2), the lobes of the perianth are exactly opposite: two outer and two inner ones, but there are apparently six stamens. Of these the four outer ones actually represent only two duplicated, which explains their position, while the two inner ones are exactly opposite the inner lobes of the perianth. The floral diagram of *P. virginianum*, as shown in fig. 2, thus corresponds with that of *Oxyria digyna* according to EICHLER (6),

with the exception, however, that in our *Polygonum* the arrangement of the floral organs is tangential instead of radial to the main axis. Moreover, in *P. virginianum* the anthers of the outer stamens are introrse, those of the inner ones extrorse, while in *Oxyria* all the anthers are introrse. This diagram of *Polygonum* recurs in many other species of the genus, according to EICHLER. It is not the only type of diagram which we have observed, however, since many flowers have only five stamens, the posterior being undeveloped; in the last flower to open we noticed almost constantly six stamens. Nectaries in the shape of small glands (fig. 4) alternate with the stamens. The perianth is somewhat unequally four-parted, the two outer lobes being a little shorter than the inner ones; the two inner stamens are a little longer than the others. At the time of pollination all the anthers are open, and the two styles are somewhat shorter than the stamens, although with the minute stigmata viscid, capable of receiving the pollen. After pollination has been effected the parallel styles increase considerably in length (fig. 5), become rigid and deflexed, forming an obtuse angle with the ovary, and with the summits recurved, bearing the minute stigmata. The perianth persists, tightly inclosing the achenium, and falls off at maturity.

According to MUELLER (15) the flowers of some species of *Polygonum* are adapted to pollination by insects, for instance *P. bistorta*, in which the stamens at the time of flowering are distinctly longer than the perianth, while the styles do not attain that length before the stamens have withered completely and dropped off (proterandrous dichogamy). In *P. viviparum* the flowers vary from bisexual, proterandrous, to purely female, but in *P. persicaria* and *P. lapathifolium* the pollination fluctuates between self and cross-pollination, since the flowers are bisexual, relatively small, and secrete very little nectar. Although the flowers of *P. minus* Huds. are not smaller than those of *P. persicaria*, they are nevertheless much more seldom visited by insects, owing to the loose, thin inflorescence, making the flowers less conspicuous than in the dense flowered spikes of *P. persicaria*.

In *P. virginianum* the flowers are constructed in about the same manner as those of *P. persicaria* and *P. minus*, thus being adapted to self or cross-pollination. Self-pollination seems undoubtedly to be

the more frequent, since the flowers are very inconspicuous, being small, and the inflorescence very lax. The three-flowered rhipidia are very remote, and there is almost a month between the opening of the first and the last developed flowers. No visitors were observed, nevertheless mature fruits were found in many specimens. The singular structure of the two persistent, rigid, hooked styles facilitates the dispersal of the fruit by means of animals, and at the same time the stoloniferous habit enables the plant to spread over a larger area. The species seems to be very particular with regard to the nature of environment; however, I have never found it outside shady woods. Some specimens, transplanted to the open along a creek, failed to produce flowers and lived only one year.

INTERNAL STRUCTURE OF VEGETATIVE ORGANS.—As stated, an account will be given of the anatomical structure of the section *Tovara*, as represented on this continent, besides some species of the sections *Persicaria*, *Avicularia*, and *Cephalophilon*, in order to show the probable correlation between structure and environment.

Polygonum virginianum L.—The roots are of the nutritive type, being very slender and of relatively short duration. Secondary tissues appear early, but are confined to the development of a narrow zone of secondary cortex surrounding a stele, of which the greater portion is occupied by numerous strata of thick walled conjunctive tissue, arranged very regularly in radii and extending to the center. The stele is frequently heptarch, but with only a few wide vessels in each ray. No cork was observed, and the endodermis as well as the primary cortex persists for some time, but more or less collapsed.

The stolons, at the stage of those in fig. 1, are cylindrical and glabrous; the cuticle is thin, smooth, and the outer cell wall of the epidermis is slightly thickened. The cortex consists of 8–10 compact, homogeneous strata, filled with starch, and the hypodermal layer becomes a phellogen. There is no endodermis, but an almost closed sheath of stereome, about three layers and moderately thick walled. The stele contains a single band of many collateral mestome strands, and between these an interfascicular cambium has developed some few strata of libriform, but of libriform only, since no purely leptomatic strands appear in the subterranean stem portions. The libriform forms a closed sheath between the inner flank of the hadrome

and the periphery of the pith, which is compact, solid, not hollow, filled with starch, and some cells contain a brown gelatinous substance.

By the time the stolons are in their second year of growth and have produced an aerial shoot, they have increased considerably in thickness, corresponding with the thick stem portion from which they have developed (fig. 1). The peripheral tissue is now cork, relatively thick walled, and in five or six strata, surrounding the primary cortex and stereomatic pericycle. Inside of this a secondary cortical parenchyma has developed, consisting of about ten strata, filled with starch, and several cells containing a brown gelatinous substance, as observed in the stolons. The stele now shows a band of separate leptome strands in the same radii as the stereome strands of the pericycle, several strata of cambium, some deep, but very narrow rays of hadrome and secondary libriform. The interfascicular tissue is represented by broad rays of starch bearing parenchyma extending from the pith to the secondary cortex. The pith is compact, thin walled, and contains much starch, and the gelatinous brown substance is visible in many cells. The increase in thickness has thus been effected by the activity of the intrafascicular and interfascicular cambium developing a secondary cortex, secondary mestome, and libriform and medullary rays at the expense of most of the interfascicular libriform, which has become more or less obliterated. Also, the formerly closed pericycle has become divided into separate arches of stereome.

In the aerial stem the internodes between the large green leaves are cylindrical and glabrous. The cuticle and the epidermis show the same structure as in the stolons, but no phellogen appears; no stomata were observed. Collenchyma is well represented as a continuous, hypodermal zone of about four strata surrounding the cortex proper, which consists of four or five layers of chlorophyll-bearing parenchyma, with relatively wide, rhombic intercellular spaces. Several of the cells contain aggregated crystals of calcium oxalate, and the brown gelatinous substance mentioned was also noticed in some of the cells. There is a large celled endodermis, of which several cells have become differentiated into long tubular reservoirs containing tannin of a clear, brownish color. A closed sheath of thick walled

stereome in three or four strata surrounds a stele of a single band of separate, collateral mestome strands alternating with some very thin strands of pure leptome (fig. 13). Between these mestome strands are layers of libriform developed from the interfascicular cambium. The pith is thin walled, not hollow, and contains starch and aggregated crystals of calcium oxalate. Tannin reservoirs of great length are scattered in the pith (figs. 11, 12).

A somewhat modified structure is to be observed in the swollen nodes. In these the complete cortex is collenchymatic, about fifteen layers, and very narrow in proportion to the large pith. The endodermis is thin walled, and a few of the cells are tannin reservoirs. There is no stereomatic pericycle, the mestome strands bordering directly on the endodermis. The structure of the mestome is as described, but the libriform (inter as well as intrafascicular) is very thin walled. Purely leptomatic strands are much less frequent than in the internodes. The pith is thin walled, and contains numerous aggregated crystals of calcium oxalate, besides tannin.

The very thin internodes of the inflorescence are cylindrical and glabrous; the epidermis is thick walled, almost collenchymatic; and the cortex consists of about ten strata of parenchyma with a little chlorophyll. There is no endodermis, but a closed sheath of thick walled stereome in six or seven strata surrounds a band of many collateral mestome strands alternating with some which are purely leptomatic. The narrow pith is thin walled, starch-bearing, and contains also tannin.

In comparing the structure of the subterranean internodes with the aerial, the development of secondary tissues in the former naturally results in cork and a secondary cortex in the subterranean, while in the aerial internodes the growth of the inner tissues does not interfere with the preservation of the peripheral tissues. While the mechanical tissue, the stereome, occurs as a sheath around the mestome strands in both stems, the collenchyma is confined to the aerial, representing the whole cortex in the nodes. Another difference depends upon the non-development of purely leptomatic strands in the subterranean internodes, besides the absence of tannin reservoirs.

The structure of these internodes, subterranean and aerial, thus illustrates a case where some of COSTANTIN'S (5) conclusions cannot

be accepted, at least not as a general rule. According to this author the subterranean stem should be destitute of stereome, in the form of a pericycle, and as strands encircling the hadrome. The cambium, intra and interfascicular, should be less active, and the pith should be narrower than the cortical parenchyma. As already described, stereome is well represented in the subterranean stem; the cambium is active by developing a secondary cortex and secondary libriform; and the difference in width of the pith is but very slight. On the other hand, the development of cork, the disappearance of the collenchyma, and the increase in width of the cortical parenchyma are structures characteristic of the subterranean stem of our *Polygonum*, and are in accordance with the observations made by COSTANTIN in dicotyledonous plants. If this author had extended his studies to the Monocotyledones, he would not have failed to notice the very strong development of the stereome in the stolons of numerous Gramineae and Cyperaceae, representing an epharmonic character of great importance.

The relatively ample leaves are held in a horizontal position, and the structure is bifacial. The cuticle is thin and smooth; the epidermis on the upper face is developed as large papillae all over the blade (fig. 7); while on the lower face the cells show the ordinary structure and are much smaller; the papillae are slightly thick walled. Stomata (fig. 10) abound on the lower face, raised somewhat above the adjoining epidermis, and the air chamber is very wide. Pointed, pluricellular hairs (fig. 6), papillose at apex, are frequent along the veins on the dorsal face. The chlorenchyma is composed of a single layer of short palisade cells covering an open pneumatic tissue of about three strata; large idioblasts containing aggregated crystals of calcium oxalate abound in the chlorenchyma close to the epidermis of both faces. A very characteristic structure is exhibited by the midrib; there are no papillae, the epidermis cells being small and thick walled on both faces. Some few strata of hypodermal collenchyma on the dorsal face, and a large strand on the ventral inclose a large, thin walled water storage tissue with some aggregated crystals. In the center of this tissue is a circular (in cross-sections) band of five or six collateral mestome strands, all turning the hadrome inward, and each strand is supported by an arch of stereome, not very thick

walled. The lateral veins of first order show the same structure, but contain only one mestome strand; the thinner veins of fourth or fifth order are surrounded by thin walled parenchyma sheaths, inside of which some few stereids are located on the leptome side. Tannin reservoirs were observed in the center of the stele in the midrib, as well as on the leptome side.

The leaves of the stolons are membranaceous, short, and tubular; the thin walled epidermis is perfectly glabrous, and there are no stomata and no water pores. There are about six layers of parenchyma in the middle of the leaves, containing a little starch, and some cells containing a brown gelatinous substance, especially in those close to the mestome strands, which are located in a single plane. They contain mostly leptome supported by a few stereids, but have no parenchyma sheaths.

The papillose ventral epidermis, papillose hairs, and midrib containing a stele are features characteristic of the aerial leaves of this species. Furthermore, the absence of the dark horseshoe-shaped spot upon the upper face of the leaves, so characteristic of the species of *Polygonum*, deserves attention. As a matter of fact this spot does appear upon the first developed leaves, which are unfolded before the locality becomes shady, but disappears soon.

SECTION PERSICARIA: *Polygonum pennsylvanicum* L., *P. lapathifolium* L., and *P. hydropiperoides* Michx.—The leaves show a very uniform structure in these species. They are unifacial with regard to the distribution of the stomata, bifacial with reference to the chlorenchyma, a typical palisade tissue of mostly two strata being well developed. Large idioblasts containing aggregated crystals of calcium oxalate were observed in the palisade tissue of *P. pennsylvanicum*, in the pneumatic tissue of *P. hydropiperoides*, and also in the ventral epidermis of the third species. Sessile glandular hairs with the head pluricellular were observed on both faces of the leaf in *P. lapathifolium*, but only on the dorsal in the other two species. Pluricellular papillose hairs like those of *P. virginianum*, but a little shorter, occur on the dorsal face of *P. lapathifolium*. The midrib contains a stele of six or seven separate collateral mestome strands in all three species, imbedded in a large strand of thin walled parenchyma, a water storage tissue. Tannin reservoirs and aggregated crys-

tals were observed in this tissue. There are a few strata of hypodermal collenchyma on both faces of the midrib, and an arch of rather thin walled stereome covers the leptome of the mestome strands in all the species.

In the stem of *P. hydropiperoides* the outer cell wall of the epidermis of the internodes forms numerous, very distinct, longitudinal crests, covered by the thin cuticle, a structure not observed in the other species. A hypodermal collenchyma of three or four separate strands occurs in *P. lapathifolium*, while in the other species this tissue forms a continuous sheath around the cortex proper, which is thin walled and destitute of chlorophyll. There is no distinct endodermis, but a closed stereomatic pericycle of one to three strata surrounding the stele. This contains a single band of many thick, collateral mestome strands alternating with some which are purely leptomatic. The pith is thin walled, starch-bearing, hollow in *P. lapathifolium*, solid in the others. Aggregated crystals of calcium oxalate were observed in the pith of all three species, but no tannin.

Characteristic of the nodes is the high development of the hypodermal collenchyma, being very thick walled and of many layers, about twelve in *P. pennsylvanicum*, and about eight in the other species. It represents the entire cortex in *P. pennsylvanicum* and *P. lapathifolium*, while in the third species the interior part of the cortex is thin walled, parenchymatic, and of three or four strata. No endodermis occurs in *P. lapathifolium*, but in the two others. It is thin walled and contains starch. While a very thin walled pericycle was observed in *P. pennsylvanicum*, this tissue is absent from the other species. The stele shows the same structure as the internodes, but the libriform is thin walled. The pith is large and contains starch in all the species; it is hollow in *P. lapathifolium*, solid in the others. Tannin was observed in the cortex and pith of *P. hydropiperoides*, none in the other species. Aggregated crystals were found in the pith of *P. pennsylvanicum* and *P. hydropiperoides;* sphaero-crystals in the pith of *P. lapathifolium*. These sphaero-crystals are quite abundant in the pith of the internodes and nodes, and they were also observed by SCHMIDT in some species of *Polygonum*. My material had been kept in alcohol for several months, and the crystals showed exactly the same structure as those of inulin, so common in the Com-

positae, and extended from one cell to another. They were soluble in dilute acids, partly so also in an aqueous solution of potassic hydrate, but water heated to 50° C. failed to dissolve them.

The peduncle of the inflorescence is glabrous in *P. hydropiperoides*, but the outer cell wall of the epidermis shows here also the sharp longitudinal crests. In the other two species the peduncle is very hairy, with the same types of hairs as observed in the leaves, long stalked glandular (fig. 9) or pointed papillose. The hypodermal collenchyma occurs as separate strands in *P. lapathifolium*, but as a continuous tissue in the two others; the inner three to seven layers of the cortex are thin walled in all the species, and in *P. hydropiperoides* this parenchyma is rich in aggregated crystals of calcium oxalate, besides many cells containing a brownish gelatinous substance. No endodermis was observed in *P. hydropiperoides*, but was in the two others, where several of the cells represented tannin reservoirs. A thin walled continuous pericycle surrounds the stele in all three species, and the structure of the stele is as previously described, including the very thin, purely leptomatic strands. Tannin reservoirs were found in the pith of all the species, besides aggregated crystals in *P. lapathifolium*.

SECTION AVICULARIA: *Polygonum aequale* Lindm. and *P. erectum* L.—*P. aequale* has recently been established by LINDMAN (11), in a paper on the so-called *P. aviculare* L., in which he divides this species into *P. aequale* and *P. heterophyllum*, in accordance with the material which this author has studied in the herbarium of CLIFFORT (Mus. Brit.), and in the herbarium of LINNAEUS, but labeled *P. aviculare* L. They are very distinct species according to the diagnoses and the excellent figures. Heterophylly is characteristic of the former, while the shape of the leaves is uniform in the latter; the perianth is almost cleft to the base in the former, while in the latter the lobes are much shorter (figs. 17, 18), and frequently even shorter than the basal, tubular part of the perianth. In the former the perianth is generally larger than the mature nut, but of the same length or a little shorter in *P. aequale*. The color and structure of the surface of the nut are more constant in these two species than the outline, being brownish or more or less blackish and dull with longitudinal striae in *P. heterophyllum*, and black or blackish, more or less shining, in-

distinctly striate or punctulate in *P. aequale*. Abnormal fruits are not seldom to be observed in the late fall, where the nuts are attenuated into a long point, and considerably longer than the perianth, besides being much narrower and of a pale greenish or yellowish color (fig. 19).

A third species, *P. calcatum*, has been described by the same author (12), but this is not to be considered as a variety of the old *P. aviculare*, but as a parallel species like *P. raji*, *P. bellardi*, etc. Characteristic of *P. calcatum* is the perianth, forming an oblong solid foot beneath the nut. The number of stamens is five, and the nut black, smooth, and shining. It is a native of Sweden, Lapland, Germany, southern Russia, western Asia, and the Himalayas; the other two species are evidently cosmopolitan. I have given this account of these plants since they are evidently widely distributed on this continent, and ought to be recorded as distinct species, as proposed by LINDMAN, instead of collective, so-called *P. aviculare* L. With reference to *P. aequale*, this is the species that occurs in the vicinity of Washington, D.C., along roads, especially in sandy or clayish soil in the open. *P. erectum* grows in gravelly soil in the open.

The leaves are unifacial in both species, not only with reference to the stomata, but also in regard to the chlorenchyma, representing a ventral and a dorsal palisade tissue. The leaves are held in a vertical position in *P. erectum*, partly also in the other species. No hairs were observed, and the epidermis is not papillose. In *P. erectum* the chlorenchyma consists of one layer of high palisade cells on both faces of the blade, inclosing a pneumatic tissue of roundish cells; in *P. aequale* there are two layers of high palisade cells on the ventral, and one to two strata, somewhat lower, on the dorsal face. Large idioblasts with aggregated crystals of calcium oxalate are frequent, especially in the dorsal palisade tissue of both species. Hypodermal strands of collenchyma were observed above and below the midrib, and also as arches covering the leptome of the mestome strands in *P. erectum*, while in the other species the collenchyma is confined to the dorsal face of the midrib. Four collateral mestome strands, arranged like a stele, and with the hadrome turned inward, constitute the midrib in *P. erectum*, while in *P. aequale* there is only one median mestome strand. The water storage tissue of the midrib is large in the former species and

very small in the latter. Tannin reservoirs form a closed sheath around the stele in *P. erectum*, but are absent from the other species. While no stereome occurs in *P. erectum*, a layer of this tissue covers the leptome of the midrib in *P. aequale*.

Some specimens of *P. erectum*, which grew in the shade (completely covered by a dense vegetation of very tall *Solidago*, *Erigeron*, and *Lactuca*) showed a somewhat modified structure, when compared with the plant from the open. The leaves were held in a horizontal position; the outer cell wall of the epidermis was slightly raised on both faces, although not papillose; the palisade tissue was less typical and less compact; and the collenchyma was less thick walled and not well represented. Finally, the water storage tissue was less developed, and did not contain any tannin reservoirs.

The internodes of the stem are glabrous in both species. While typical collenchyma is so well represented as hypodermal strands in *Polygonum* in general, in the section *Avicularia* a somewhat peculiar structure was observed. In *P. erectum* the hypodermal collenchyma is replaced by stereome, of which the cells, in cross-sections, show a very distinct interior ring bordering upon the lumen (fig. 15). When treated with chlor-zinc-iodine, however, the entire cell wall attains the same bright yellow color as the stereomatic pericycle and libriform in the same section. In *P. aequale*, on the other hand, the structure of this hypodermal tissue resembles more collenchyma in cross-sections, with the only exception of the cell wall showing a similar internal ring as in the preceding species. Viewed in longitudinal sections, however, the cells are long, and attenuated at both ends. Treated with chlor-zinc-iodine, the cell walls of the various strata, peripheral and internal, show a different reaction. In the three or four peripheral strata, the outer part of the cell wall becomes deep purple, while the internal ring remains bright yellow. In the interior three or four strata of this same tissue the complete cell wall becomes deep purple; in other words, the cells of the peripheral strata of this tissue are not purely collenchymatic, while those of the interior strata are so. The stereome covering the leptome of the collateral mestome strands, as well as the intra and interfascicular libriform, showed the typical reaction of true stereome in this species. This peculiar structure was observed and described as characteristic of *Poly-*

gonum "aviculare" by RÜTZOU (**19**), and the material examined, having been collected in Denmark, was evidently either *P. aequale* or *P. heterophyllum*.

When following the structure of this hypodermal tissue farther down toward the node, the reaction becomes more collenchymatic in all the cells, and the node itself shows this reaction in all the strata. The other part of the cortex in *P. erectum* is developed as a palisade tissue of one or two layers, surrounding some few strata of round cells rich in starch, and many of these contain the brownish gelatinous substance. In *P. aequale* the cortical parenchyma is of three or four strata of isodiametric cells, here and there interspersed with palisade cells, placed obliquely to the surface, and destitute of starch. There is a distinct endodermis in *P. aequale*. A stereomatic pericycle surrounds the stele in the manner of separate arches on the leptome side in both species. The mestome strands constitute a single band, alternating with some purely leptomatic in *P. erectum*, while in *P. aequale* the latter type, leptomatic, is absent. The pith is solid, rich in starch, and with many cells filled with the brown gelatinous substance in *P. erectum*, while neither this substance nor starch was observed in *P. aequale*.

In the specimens of *P. erectum* from a shady situation, the palisade tissue was less typically developed, and none of the cells of the cortex contained starch, or the brown gelatinous substance; on the other hand, aggregated crystals of calcium oxalate were abundant in the pith.

Before leaving this section, attention should be called to a paper by GREVILIUS (**9**), in which an interesting account is given of the stem structure of the formerly so-called *P. aviculare*. According to this author, stomata are more numerous and the palisade cells more typically developed in the forms collected in dry sunny situations than in those from shady or damp localities; the intercellular spaces in the palisade tissue are much wider in the forms from shady or damp localities than in those from the open. In specimens "cultivated" in the shade the cells of the cortex are either isodiametric or oblong, but parallel with the axis of the stem. Crystals of calcium oxalate abound in the forms from the open, but are absent from those grown in shady or damp situations. The stereomatic tissue shows the

same development in all the forms. The hadrome contained a larger number of vessels, and with the lumen much wider in the forms from the open, dry situations than in those from damp or shady places. The pith represents the greatest width in specimens from damp or shady situations. No mention was made of the occurrence of tannin.

SECTION CEPHALOPHILON: *Polygonum arifolium* L. and *P. sagittatum* L.—The leaves of both species are unifacial with regard to the distribution of the stomata, and these are raised high above the epidermis in *P. arifolium*, but level with the epidermis in the other species. Sessile glandular hairs with the head pluricellular (fig. 14) abound on both faces of the leaf in *P. arifolium*, besides large stellate hairs (fig. 14), and this type of hair is not mentioned by SOLEREDER as occurring in the Polygonaceae. In *P. sagittatum* glandular hairs like those of the former species are distributed over the dorsal face, together with some very rigid pluricellular, pointed, and curved hairs (fig. 8). The chlorenchyma shows the same structure in both species, namely, one layer of high palisades covering an open pneumatic tissue of three strata, and large idioblasts containing aggregated crystals of calcium oxalate are scattered throughout the chlorenchyma. The midrib contains hypodermal collenchyma on both faces, and a large water storage tissue surrounds a stele of five collateral mestome strands inclosing a broad strand of parenchyma like a pith. There are several tannin reservoirs in the water storage tissue, some located near the leptome, others in the center of the stele. An arch of stereome covers the leptome of all the mestome strands.

The internodes of the stem are glabrous in *P. arifolium*, but covered with curved, pluricellular, pointed hairs in the other species. Hypodermal collenchyma in two or three continuous strata was observed in both species, but no chlorophyll-bearing parenchyma. A thin walled endodermis was observed in the apical and basal internodes of *P. arifolium*, but not in the other species. A pericycle of thin walled stereome in a few layers surrounds a band of numerous, thick, collateral mestome strands in *P. arifolium*, while in the other species there are only four thick strands, the others being very thin, but nevertheless of the collateral type. Purely leptomatic strands are numerous in both species, and located in the same band as the collateral. The pith is solid in both species, rich in starch of large

grains. Tannin was observed in the endodermis and pith of *P. arifolium*, in the pith alone in the other species.

Characteristic of the node in *P. arifolium* is the highly developed collenchyma, which represents a broad zone of about fifteen strata, besides that the pericycle is purely parenchymatic, and thin walled. Furthermore, the pith contains numerous aggregated crystals of calcium oxalate, and some few tannin reservoirs; otherwise the structure agrees with that of the internode. In *P. sagittatum* the cortex consists of a single stratum of collenchyma, and of two or three layers of thin walled parenchyma with no deposits of starch. The pericycle is also parenchymatic in this species, and the stele shows the same structure as previously described. Tannin reservoirs were present in the pith.

In the species examined, *P. virginianum* and *P. hydropiperoides* being the only perennials, the roots are all of the nutritive type, and the structure is relatively very uniform. The primary root of *P. sagittatum* from low moist ground, and *P. pennsylvanicum* from dry open fields shows the same structure, pericambial cork, but no secondary cortical parenchyma, and the stele containing much thick walled conjunctive tissue. In *P. erectum* the primary root develops not only pericambial cork, but also secondary cortical parenchyma as a broad zone of about eight strata, and in which stereome appears in the form of small isolated strands, arranged in a single band. Many cells of the cortex contain the brown gelatinous substance, and the greater mass of the stele consists of thick walled libriform. In *P. aequale* the primary cortex persists and no secondary increase takes place outside the stele. The secondary roots of *P. hydropiperoides* have a broad primary cortex of about ten strata, traversed by wide lacunae. The secondary tissues are only represented by homogeneous, thin walled strata of cork, developed from the pericambium, and by the stele, in which thick walled conjunctive tissue is much in evidence. No tannin reservoirs were observed in any of these roots, and no aggregated crystals.

Discussion

When comparing the leaf structure of these species of *Polygonum*, it is noticed that *P. virginianum* is the only one in which the leaf structure is bifacial, with the stomata confined to the lower face, and

with the chlorenchyma differentiated into a ventral palisade and a dorsal pneumatic tissue. On the other hand, a unifacial structure as to the distribution of the stomata and the development of palisade cells in the ventral and the dorsal part of the blade is characteristic of *P. erectum* and *P. aequale;* in the other species the unifacial structure depends only upon the distribution of the stomata. The papillose epidermis is characteristic of *P. virginianum* alone, but in specimens of *P. erectum*, growing in a shady situation, the outer cell wall of epidermis was slightly raised, although without forming papillae. Moreover, the absence of glandular hairs in *P. virginianum* deserves attention, since this type of hairs is so well represented in the heliophilous species except *P. erectum* and *P. aequale,* in which the leaves and stems are perfectly glabrous. The structure of the palisade cells, being relatively very short in *P. virginianum*, is characteristic of this species, but does recur in the specimens of *P. erectum* from a shady situation. In all the other species the palisade cells show the typical structure, being high and compact. With regard to the collenchyma and the water storage tissue in the midrib, however, *P. virginianum* agrees with the other species. The midrib also represents a stele of several (four to six) collateral mestome strands in all the species except *P. aequale*, which has only one. Finally, tannin reservoirs were noticed in the midrib of all the species except in *P. erectum* from the shade, and in *P. aequale*.

With reference to the structure of the internodes, the relative development of the collenchyma and the thin walled parenchymatic cortex is about the same in the various species, except that a palisade tissue was observed in *P. erectum* and partly also in *P. aequale*. Also, the hypodermal collenchyma in the former species is replaced by stereome, and in the latter by a tissue which is not purely collenchymatic. An endodermis was observed in the internodes and nodes of *P. virginianum*, as well as in some of the other species; a stereomatic pericycle was observed in all the species examined, and mostly as a continuous sheath. The structure of the stele is also very uniform, purely leptomatic strands being interspersed with collateral, except in *P. aequale*, where all the strands are of the collateral type. A typical pith is developed in all the species, frequently with deposits of starch in large grains. Tannin reservoirs were especially abundant in the pith of the

internodes and nodes of *P. virginianum*, but only in the aerial; they occur also in the endodermis. In most of the other species tannin was noticed in the endodermis, the pericycle, and especially in the pith, but was absent from *P. erectum* in the shade, and also from *P. aequale*. Aggregated crystals of calcium oxalate were quite abundant in the cortex and pith of the aerial internodes of *P. virginianum*, and also in the pith of *P. erectum* from the shade; while they were absent from the typical specimens collected in the open. No crystals were observed in any part of the stem of *P. aequale*. The presence and distribution of crystals of calcium oxalate may not be constant, however, since GREVILIUS found these only in stems of *P. "aviculare,"* growing in the open and fully exposed to the sun; they appeared to be totally absent from specimens grown in the shade or in wet ground. In my material of *P. aequale*, collected in the open, no crystals were observed; on the other hand, they were quite abundant in *P. arifolium*, but entirely absent from *P. sagittatum*, although both species are inhabitants of bogs, wet places, etc.

According to SOLEREDER (**20**) only a small number of species of *Polygonum* are known from an anatomical point of view, but apparently the structure of the vegetative organs is very uniform. Among the characters recorded in the preceding pages, two appear to be new, namely, the stellate hairs in *P. arifolium* and the papillose epidermis in *P. virginianum*. Regarding the root structure, which is not mentioned in SOLEREDER'S work, nothing of importance was found except that the secondary cortex contains separate strands of typical stereome in *P. erectum*.

While the section *Tovara* is monotypic on this continent, a second species, *P. filiforme*, is credited to Japan. THUNBERG, however, did not describe the very important fruiting stage, and from the brief characterization of the section given by DAMMER it is not certain whether the Japanese species is distinct from the American, or merely representing a form of this. BENTHAM and HOOKER considered them closely allied. As a section *Tovara* is quite distinct from the others, notwithstanding the fact that the floral structure, the four-parted perianth, the number of stamens fluctuating between four and six, and the two styles recurs in the section *Tephis* Adans. This section, however, is closely related to *Avicularia*, hence the

habit is entirely distinct; moreover, the final structure of the styles is very different. Concerning the internal structure of the vegetative organs, *Tovara* possesses some truly epharmonic characters, expressed by the papillose leaf epidermis and the short, plump palisade cells, none of which occur in the heliophilous species examined. The peculiar hairs, the large idioblasts containing aggregated crystals of calcium oxalate, the tannin reservoirs, and the midrib of the leaf representing a stele may be regarded as simply fixed, since they recur in the heliophilous species of the genus. When we compare the structure of *P. virginianum* with that of the specimens of *P. erectum*, grown in the shade, the analogy points toward the possibility that the characters they have in common are epharmonic, and especially adapted to shady situations. With regard to the species from the open, the unifacial leaf structure with palisade cells on both faces, as represented by the section *Avicularia*, corresponds well with the foliage being held in an erect or at least ascending position, thus fully exposed to the light, and is evidently an epharmonic character. In the other species the horizontal position of the leaves is accompanied by a dorsiventral structure of the chlorenchyma, as is generally the case. Such structures as the stellate hairs in *P. arifolium*, however, the crested epidermis in *P. hydropiperoides*, and the stereomatic collenchyma in *Avicularia* cannot be accounted for, except as purely specific structures.

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EXPLANATION OF PLATES I, II

Polygonum virginianum

PLATE I

FIG. 1.—Subterrannean stem with stolons and one aerial shoot (*St*); natural size.

FIG. 2.—Diagram of flower: *R*, main axis.

FIG. 3.—Flower; enlarged.

FIG. 4.—Two perianth lobes with stamens; enlarged.

FIG. 5.—Fruiting stage; nut inclosed within persisting perianth; enlarged.

FIG. 6.—Hair from dorsal face of leaf; ×320.

FIG. 7.—Cross-section of leaf: *Ep*, epidermis; *P*, palisade tissue; ×320.

FIG. 8.—*P. sagittatum:* hair from leaf; ×240.

FIG. 9.—*P. pennsylvanicum:* hair from apical internode beneath inflorescence; ×360.

PLATE II

FIG. 10.—Dorsal epidermis of leaf with stoma; ×320.

FIG. 11.—Longitudinal section of pith of internode, showing tannin reservoir and cells with aggregated crystals of calcium oxalate; ×240.

FIG. 12.—Cross-section of pith of internode, showing two tannin reservoirs and two cells with crystals; ×240.

FIG. 13.—Cross-section of part of middle internode: *St*, steromatic pericycle; *L*, interfascicular cambium and libriform, and strand of pure eptome; *P*, pith; ×320.

FIG. 14.—*P. arifolium:* glandular and stellate hair from dorsal face of leaf; ×480 and 360.

FIG. 15.—*P. erectum:* cross-section of peripheral part of internode; *Ep*, epidermis; *C*, cortex; *St*, stereome; ×480.

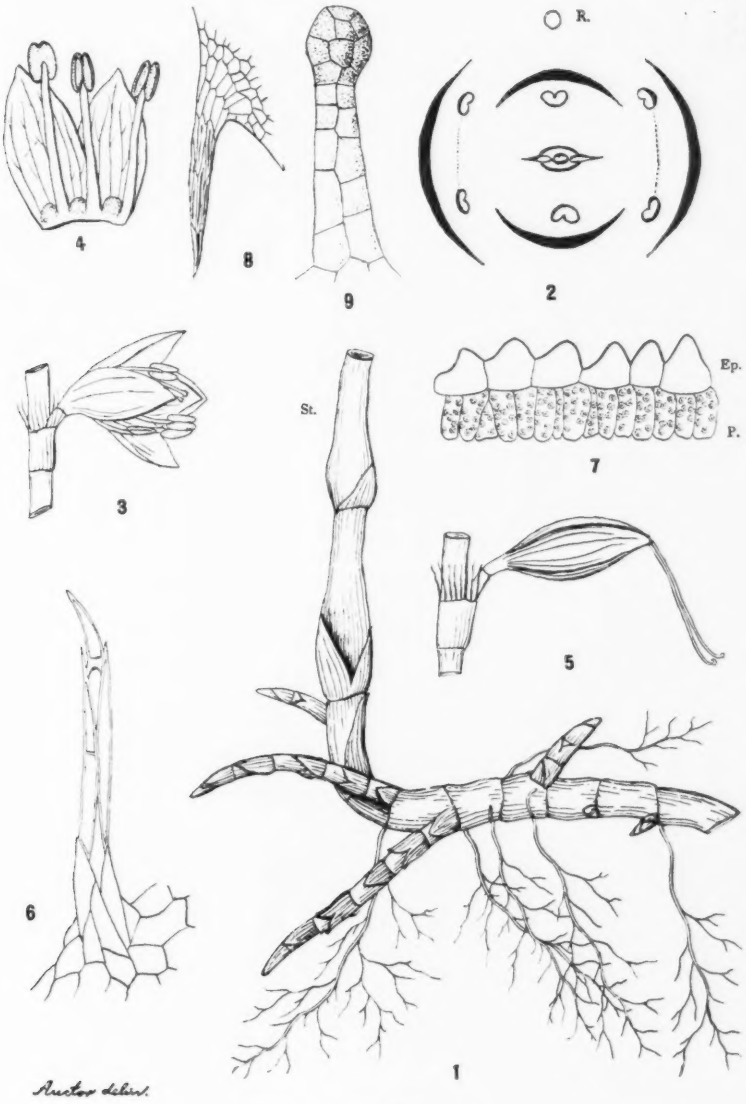
P. aequale

FIG. 16.—Leaf; twice natural size.

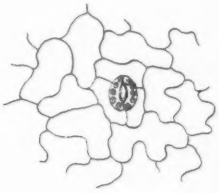
FIG. 17.—Flower bud; ×8.

FIG. 18.—Mature flower with nut hidden within persisting perianth; ×8.

FIG. 19.—Abnormal flower with fruit extending beyond perianth, collected late in fall; ×8.



HOLM on POLYGONUM



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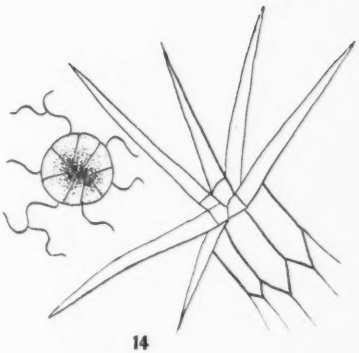

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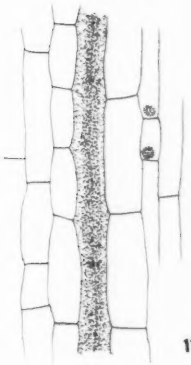

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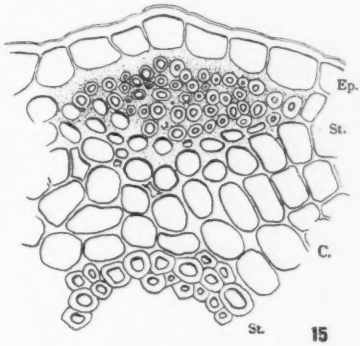

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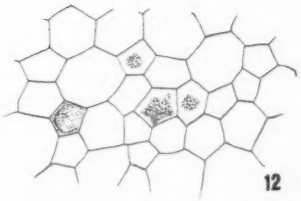

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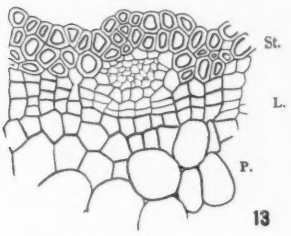

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Auctor delin.

HOLM on POLYGONUM

SEXUAL DIMORPHISM IN MUCORALES

I. INTRASPECIFIC REACTIONS

A. F. Blakeslee, J. L. Cartledge, D. S. Welch, and A. D. Bergner

Introduction

In a previous paper (7) experimental evidence for a strict sexual dimorphism in *Cunninghamella* has been given in some detail. The literature bearing on the question of sexuality in the Mucors is there cited, especially as it offers evidence in regard to sexual dimorphism in this group of fungi. In a still earlier publication (5) detailed tests are given for a single species of *Absidia*, as well as a provisional summary of tests with other forms. The methods employed in collecting the races studied, making the tests, and tabulating the reactions have already been described (6). The purpose of the present paper is to give in a series of tables the results of testing races within species other than those already recorded. In a second paper in this issue are given the results of testing together races belonging to different species.

Most of the tests recorded were made during the years 1919–1923. Unless otherwise noted, the cultures were run at laboratory temperature on our standard nutrient, no. 230, consisting of 2 per cent each of agar, dry malt, and dextrose, with 0.1 per cent peptone. Other nutrients used were no. 360, consisting of no. 230 plus 0.5 per cent dried horse dung decoction; no. 362, consisting of agar with addition of 2 per cent whey powder plus 1 per cent dextrose; no. 388, consisting of agar with 6 per cent malt and 1 per cent dextrose; no. 391, consisting of 3 per cent dextrose with 1 per cent dried horse dung decoction.

In the tables the letters A to D are used to indicate the different strengths of sexual activity measured by the relative number of zygospores produced in the given contrasts. Each race has been given a final numerical grade, made up of the average of its reactions with the testers of opposite sex (grade A having the numerical rank 4,

and grade D a rank of 1), and the races have been arranged in the tables according to their final grading. Since the various species differ in the abundance of zygospore production, the grades are comparable only within a given species. A race which has failed to show a sexual reaction with any of the testers has been classed provisionally as "neutral." It has been shown (**2**), however, that the races listed as neutral may be merely sexually weak races which have not been contrasted with sufficiently strong testers to elicit a sexual reaction. In cases in which the scarcity of zygospores or their position in the culture suggested the possibility of infection, the contrasts have been repeated. Contrasts between races with like signs are not represented in the tables, since no reactions have been found in such combinations.

The sources for the *Mucor* cultures indicated as T and H numbers in the various tables may be found in our earlier paper (**7**), except T 43 from a squirrel's nest in Cold Spring Harbor; T 71 from orange skins, Cold Spring Harbor; and H 3 from Brazil nuts from Huntington, New York. Where no T number is given, the origin of the culture may be found in the text. Several of the races studied were obtained from the Centralstelle für Pilzkulturen (**1**).

Experiments

ABSIDIA BLAKESLEEANA Lendner.—This species, recently described by LENDNER (**11**), has been discussed in an earlier paper (**5**) under the provisional designation "dark" *Absidia*, and a table has been given showing the strengths of reactions between the different races. Nineteen races were found to be (+), 18 (−), and 3 neutral. All the 40 races there listed were obtained from the same Brazil nuts which furnished the *Cunninghamella bertholletiae* previously reported (**7**), except race no. 638 which was supplied by Dr. THAXTER from a culture from Panama. Contrasts were run at oven temperature of about 30° C.

ABSIDIA CAERULEA Bainier.—Of the cultures listed in table I, races 911 and 912 were isolated from a culture producing zygospores on rabbit dung collected from Mt. Katahdin, Maine, in 1902; races 913 and 914 were supplied by the Centralstelle as mated strains under the name of *A. orchidis* and listed as coming from HAGEM; races 434–445 and 531–537 were isolated from ten samples of soil from

different localities in Cold Spring Harbor. All the 231 possible combinations were made with the twenty-two races. As shown in table I, four races were (+), thirteen were (−), and five (436, 437, 440, 441, and 443) not shown in the table were neutral. The cultures were run at about 26° C.

ABSIDIA CYLINDROSPORA Hagem.—The two races of this species were secured from the Centralstelle and were listed as coming from Hagem. The zygospores have been obtained on our nutrient 362 at a temperature of about 24° C.

ABSIDIA DUBIA Bainier.—This *Absidia*, which had been provisionally listed by us as "Cymose" *Absidia*, has been kindly determined by Professor LENDNER as probably *A. dubia*. The zygospores

TABLE I

ABSIDIA CAERULEA

GRADE	(+) RACES	(−) RACES												
		533	534	912	914	532	535	434	445	531	439	444	435	442
2.31.....	913	B	C	B	B	B	C	B	C	C	C	B	D	D
2.23.....	911	A	B	A	B	B	B	C	C	C	C	D	O	O
2.07.....	536	B	B	C	B	C	B	C	B	C	C	C	O	O
1.54.....	537	C	B	C	C	C	C	C	D	C	D	D	O	O
Grade..	.....	3.00	2.75	2.75	2.75	2.50	2.50	2.25	2.00	2.00	1.75	1.75	0.25	0.25

are more or less spherical, and generally show either an equatorial ridge or more frequently a band which may have a width of about one-third of the zygospore. All our races were obtained from Brazil nut cultures, as indicated by the T and H source numbers (7). Difficulty was encountered at first in obtaining zygospore formation, and at the best the species does not seem to possess strong sexual activity. On account of the difficulty in distinguishing the races of this species from certain closely allied types, only races previously sexed by the imperfect hybridization reaction with other species were used. In consequence no neutrals appear in table II. Of the twenty-four races tested, four were (+) and twenty were (−). The cultures were run on nutrient 388 at about 30° C.

ABISIDIA GLAUCA Hagem.—Races 666 and 669 were isolated from the same sample of forest soil from Cold Spring Harbor; 917 and 918 were isolated by one of us from a zygosporic culture in the Harvard

Laboratory, and, as mated strains, have been kept under cultivation for about twenty years; 915 and 916 were supplied by the Centralstelle as mated strains of *A. glauca* and listed as coming from HAGEM; 919 was from the Centralstelle under the name *A. septata* and listed as coming from LENDNER; 920 was from the Centralstelle under the name *A. glauca* var. *paradoxica* and listed as coming from NAMYSLOWSKI. The cultures from the Centralstelle are here reported under the names they bore when received by one of us, and their origin is attributed to the authors later listed in the report of the

TABLE II

ABSIDIA DUBIA

SOURCE		T43B	T101B	T50E	T55E	T76A	T71A	T50K	T50g	T54C	T114A	H3	T74F1	T76B	T81A	T100	T100B	T75D	T113F	T115D	T115E
GRADE		1.00	1.00	0.75	0.75	0.75	0.75	0.50	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
		(−) RACES																			
SOURCE	GRADE	(+) RACES	597	599	376	382	383	598	375	378	379	798	206	361	384	388	390	391	596	797	800	801
T40F	1.05	374	A	O	B	D	B	C	D	O	C	O	D	O	O	O	D	D	O	D	O	D
T113A	0.65	884	O	B	O	D	O	D	O	C	O	C	O	D	O	D	O	O	D	O	D	O
T50Ec	0.15	377	O	O	O	D	O	O	D	O	O	O	O	O	D	O	O	O	O	O	O	O
T99B	0.05	389	O	D	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

Centralstelle (1) as having furnished the cultures in question. It is possible, of course, that contamination may have occurred or that errors may have crept into the records, so that the cultures originally supplied to the Centralstelle may not be, in every case, those that have been investigated by the present writers.

Table III shows the tests with *Absidia glauca*. All the forty-five possible contrast combinations were made with the total ten races. Of these four were (+), six were (−), and none failed to show a sexual reaction in at least two contrast combinations. The cultures were run at a temperature of about 26° C.

ABSIDIA RAMOSA Lendner.—Three cultures of *A. ramosa* (*typica*, var. *zurcheri*, and var. *rasti*) were obtained from the Centralstelle. The variety *zurcheri* is (−), and forms zygospores when con-

trasted with either of the two other races. The reaction between *typica* and var. *zurcheri* is stronger than that between var. *rasti* and the latter race. Cultures were run on nutrient 388 at about 33° C. Apparently the zygospores have not been reported previously. They are somewhat larger than those of *A. dubia*, being around 90×60μ, laterally compressed, with usually at least three more or less distinct ridges, one equatorial and one or two on each side of the median ridge.

ABSIDIA REPENS Van Tieghem.—A pair of (+) and (−) races of this species has been in cultivation for about twenty years. The (+) came from a laboratory culture of Brazil nuts, the (−) from nuts

TABLE III

ABSIDIA GLAUCA

GRADE	(+) RACES	(−) RACES					
		666	669	918	916	920	919
2.33	915	B	B	C	C	C	C
2.33	667	B	A	B	C	C	O
2.17	668	B	B	B	C	C	O
1.83	917	C	D	C	C	C	C
GRADE		2.75	2.75	2.50	2.00	2.00	1.00

from the Island of Margarita, Venezuela. The zygospores are without outgrowths and resemble those of a small *Mucor*. Seven races from three soil cultures at Cold Spring Harbor were contrasted with these old (+) and (−) testers, and all were found to be (+). All the reactions were B except one which was C. The cultures were run on nutrient 362 at about 24° C.

"WHORLED" ABSIDIA.—This is a species common on Brazil nuts. LENDNER, to whom material was sent for identification, writes that it corresponds to *A. cylindrospora* Hagem. Since, however, the (+) and (−) races of the latter species, obtained from the Centralstelle, form zygospores with each other but fail entirely to form zygospores with the species under discussion, it seems best, until further tests can be made with a larger collection of races of *A. cylindrospora*, to call our species provisionally "whorled" *Absidia*. All the 528 possible combinations between the total 34 races were made.

Fourteen races were (+), eighteen were (−), and two (685 and 686, from Brazil nuts obtained from Knoxville, Tennessee) were neutral, and are omitted from table IV. The cultures were run at about 27° C.

BLAKESLEA TRISPORA Thaxter.—Two mated strains of this species were kindly sent us by GEORGE F. WEBER, who had isolated them in Gainesville, Florida, from poured agar plates upon which had been planted unsterilized sclerotia of another fungus, as reported

TABLE IV

"WHORLED" ABSIDIA

SOURCE			T96	T116	T96	T90	T113	T114	T73	T114	T114	T97	T99	T66	T98	T66	T98	T79	T96	T115
GRADE			3.14	2.64	2.57	2.57	2.50	2.50	2.36	2.36	2.29	2.21	2.21	2.07	2.07	2.00	1.93	1.71	1.21	0.79
			(−) RACES																	
SOURCE	GRADE	(+) RACES		400	687	635	807	678	681	634	680	682	402	405	351	403	394	404	398	399	809
T68	2.89		395	B	B	A	A	A	A	C	B	B	B	B	B	C	B	C	B	C	D
T68	2.72		397	B	A	B	A	A	A	B	B	B	C	C	B	B	C	C	C	D	D
T52	2.61		392	A	B	B	B	C	A	C	A	A	B	C	C	B	B	C	C	D	O
T52	2.61		393	B	A	B	B	A	A	B	B	C	B	C	B	C	C	C	B	C	O
T116	2.39		684	A	C	B	A	C	C	C	B	C	C	B	B	C	B	C	C	C	D
T111	2.28		808	A	B	C	C	C	C	C	B	C	C	B	B	C	C	C	D	C	D
T96	2.22		636	A	B	C	C	C	C	C	B	C	B	C	B	C	C	C	C	C	O
T68	2.17		396	C	B	B	B	B	C	C	C	C	C	C	C	C	C	C	C	C	D
T116	2.17		688	A	C	B	B	C	C	C	C	C	B	C	C	C	B	C	D	C	D
T111	2.06		676	B	C	C	C	C	C	B	C	C	C	C	C	C	C	C	C	C	D
T113	1.89		679	B	C	B	C	C	B	C	C	C	C	C	C	C	D	C	D	O	D
T97	1.83		401	C	B	C	B	B	C	C	C	C	D	C	C	D	C	C	O	O	C
T116	1.83		683	B	C	C	C	C	B	C	C	C	D	C	D	C	O	C	C	O	D
T112	0.78		677	C	D	O	O	D	D	C	D	O	D	C	O	O	O	D	D	D	O

by him at the Kansas City meeting of the A.A.A.S. Mr. WEBER writes that he has again isolated the two strains from plated portions of cucumber leaves. Zygospores are produced in abundance on various media at laboratory temperatures.

CHAETOCLADIUM BREFELDII Van Tieghem and Le Monnier (*C. jonesii* Brefeld).—Twelve races of *Chaetocladium brefeldii* were obtained from nine different cultures of horse dung at Cold Spring Harbor. One culture was sent us by Dr. WESTON from the Harvard laboratory. We have not obtained a true imperfect hybridization between the sexual races of *Chaetocladium* and known (+) and (−) races of other mucors, although such a reaction has been reported

by BURGEFF (9). As yet, therefore, we are unable to know which sex of this parasite is (+) and which (−). In consequence, the sex of the races is not given in table V and in the summary table XIII. Possibly biochemical tests, which have not yet been made with this species, may enable us ultimately to assign the terms (+) and (−) to the races which we have under cultivation (cf. under *Parasitella*).

All possible combinations were made between the thirteen races. Five proved to be of one sex and eight of the other. The strength of reactions is shown in table V. The host used was a *Mucor* of the "*mucedo*" type, which was grown at a temperature of 15°−20°C. *Chaetocladium* is able to grow saprophytically on the usual media at laboratory temperatures, however, and to produce zygospores without a host.

TABLE V

CHAETOCLADIUM BREFELDII

GRADE	GRADE	3.00	2.60	2.20	2.20	2.00	1.60	1.20	1.00
		951	952	947	951	959	958	949	956
3.25	957	A	A	A	A	B	C	C	B
2.63	948	B	A	C	A	B	A	O	D
1.63	950	B	D	B	O	D	D	B	D
1.63	953	B	C	C	B	B	O	O	O
0.75	955	C	C	O	O	O	D	D	O

CHOANEPHORA, species A.—In 1906 Professor F. L. STEVENS of Raleigh, North Carolina, kindly gave us a tube of this undetermined species of *Choanephora* which was producing zygospores abundantly. The (+) and (−) races were isolated and kept under cultivation for several years, but eventually died out. The zygospores are similar to those of *C. cucurbitarum* but larger, measuring from 57×30 to 103×100μ, with an average of about 74×70μ. In an earlier publication (4) may be found a photograph of a culture showing the two races of this species producing a line of zygospores where they have grown in contact, and imperfect hybridization reactions with another species.

CHOANEPHORA CUCURBITARUM (B. and Rav.) Thaxter.—This *Choanephora* is commonly found growing on withered flowers or cucurbits. The races used in the tests recorded in table VI were ob-

tained from cultures of withered flowers of squash and pumpkin collected from seven different gardens within a radius of about eight miles from the laboratory at Cold Spring Harbor. In all, ten races were used as testers, including five (+) and five (−) races, and a total of 275 contrast combinations were made with the thirty-three races studied. Of these five were (+), twenty-eight were (−), and none failed to show reactions in some of the combinations. Since all of the (+) races were used as testers, it has not been necessary to

TABLE VI

CHOANEPHORA CUCURBITARUM

GRADE	(−) RACES	(+) TESTERS					GRADE	(−) RACES	(+) TESTERS				
		821	835	849	820	836			821	835	849	820	836
3.60.....	834	B	A	B	A	A	3.20...	844	A	B	B	B	B
3.60.....	843	A	A	B	B	A	3.20...	846	A	B	B	B	B
3.40.....	816	A	A	B	B	B	3.00...	823	A	C	B	B	B
3.40.....	832	A	B	A	A	C	3.00...	825	B	B	B	B	B
3.20.....	814	B	A	B	B	B	3.00...	837	B	B	B	B	B
3.20.....	819	B	A	B	B	B	3.00...	839	B	B	A	C	B
3.20.....	824	C	A	A	B	B	3.00...	840	B	B	B	B	B
3.20.....	826	A	B	B	B	B	3.00...	841	B	A	B	B	C
3.20.....	828	B	B	B	B	A	3.00...	845	A	B	B	B	C
3.20.....	829	B	A	B	B	B	3.00...	847	B	B	B	A	C
3.20.....	830	B	C	A	A	B	2.80...	818	C	B	B	B	B
3.20.....	831	B	B	A	B	B	2.80...	827	A	C	B	C	B
3.20.....	838	B	B	B	A	B	2.80...	833	B	B	B	B	C
3.20.....	842	A	A	B	C	B	2.60...	817	C	C	B	B	B
							Grade.....		3.25	3.18	3.18	3.07	2.92

group together the (−) testers (832, 823, 837, 839, and 818). An inspection of the table shows that very little difference exists between the sexual activities of the several races. It is quite possible that the slight differences in the records may be due to small variations in the environmental conditions in the different dishes, or to inconsistencies in the assigning of grades. The cultures were run on nutrient 362 at a temperature around 27° C.

CIRCINELLA SPINOSA Van Tieghem and Le Monnier.—The reactions with this species are shown in table VII. Of the races for which no T and H numbers are given, 567 and 568 came from laboratory cultures at Harvard University; 569 and 570 came from sheep dung at Cold Spring Harbor; 813 from Brazil nuts purchased at

Montreal, Canada; 905 was obtained from a squirrel nest; and 923 from horse dung at Cold Spring Harbor.

TABLE VII

CIRCINELLA SPINOSA

(−) TESTERS	(+) RACES													
	417	424	426	558	555	556	560	564	570	418	421	422	425	
413 416	C B	C C	C C	A O	D C	D C	B O	B O	C D	C O	C O	C O	C O	
Grade.....	2.50	2.00	2.00	2.00	1.50	1.50	1.50	1.50	1.50	1.00	1.00	1.00	1.00	
Source.....	T38	T51	T53	T81	T79	T80	T96	T101	–	T38	T50	T50	T52	

(−) TESTERS	(+) RACES													
	427	430	431	432	553	563	568	554	559	569	420	791	792	
413 416	C O	C O	C O	C O	C O	C O	C O	D O	D O	B –	C –	Z C	Z D	
Grade.....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50					
Source.....	T66	T74	T75	T75	T74	T101	–	T79	T81	–	T40	T117	T117	

(−) TESTERS	(+) RACES													
	780	784	785	786	787	788	789	790						
413 416	Z O	Z O	Z O	Z O	Z O	Z O	Z O	Z O						
Grade.....	.....	.....	.....	.....	.....	.....	.....	.....						
Source.....	T111	T115	T115	T115	T115	T116	T116	T117						

(+) TESTERS	(−) RACES													
	416	413	419	429	433	415	428	557	562	414	781	794	782	783
426 417	C B	C C	C C	C C	D C	C O	C O	C O	O C	D O	Z B	Z B	Z C	Z O
Grade.....	2.50	2.00	2.00	2.00	1.50	1.00	1.00	1.00	1.00	0.50	.....	.....	.....	.....
Source.....	T27	H2	T39	T66	T76	H2	T66	T80	T99	H2	T111	T118	T112	T113

Of the total fifty-five races, thirty-four were (+), fourteen were (−), and seven races (412 (H_2), 423 (T 51), 567, 793 (T 117), 813, 905, and 923) which do not appear in the table, were neutral. Races 569 and 420 were lost before the second test was made. Races 780–

792 and 794 were tested in the first series, but their zygosporic reactions (Z) were not graded. The cultures were run at a temperature of about 26° C. on nutrient 362. In the tests with 416 and 417, the nutrient was modified by the addition of 0.1 per cent lactic acid, which had little if any influence upon the growth of the cultures. It was found necessary to inoculate the races very close together (1–2 mm. apart), since zygospores fail to form at any considerable distance from the points of inoculation.

CIRCINELLA UMBELLATA Van Tieghem and Le Monnier.—A pair of races of this species has been kept under cultivation for about twenty years. The (+) race came on a culture of rat dung from China, the (−) from a culture of dog dung from Cambridge, Massachusetts. Zygospores have been obtained on nutrient 362 at a temperature of about 24° C.

CUNNINGHAMELLA.—In our earlier paper (7), a detailed report was given of sexual reactions within the species *C. bertholletiae* Stadel, *C. echinata* Thaxter, *C. elegans* Lendner, and a form provisionally listed as *Cunninghamella* A.

HELICOSTYLUM PIRIFORME Bainier.—Races 860 and 861 were found on cultures of Brazil nuts purchased in Oyster Bay, New York, and New York City respectively. Races 862–865 and 869 were obtained from dung cultures from Cold Spring Harbor. Races 867 and 868 were from laboratory cultures and have been kept running for a number of years. The zygospores resemble those of *Mucor mucedo*, but are smaller. Uncoiled outgrowths resembling mycelial filaments may arise from the suspensors and extend their growth to a considerable distance. All of the thirty-six possible contrast combinations were made with the total nine races. Of these six were (+), three (−), and none was neutral. All the contrasts between (+) and (−) races gave either B or C reactions. Cultures were run on nutrient 362 at temperatures between 23° and 26° C.

MUCOR GRISEO-CYANUS Hagem.—Of the races of this species tested, a (+) came from the Harvard laboratory, and another of the same sex was obtained from a decayed pumpkin; of the (−) races, two were obtained from rabbit dung and one from a soil isolation culture from Cold Spring Harbor, two from decayed raspberries from Amherst, Massachusetts, and one from the Centralstelle. Of

the total nine races tested, three were (+) and six were (−). Ten combinations were made, and all the contrasts between (+) and (−) races gave either B or C reactions. Cultures were run on nutrient 362 at about 25° C.

MUCOR HIEMALIS Wehmer.—One (+) and one (−) race of this species were obtained from the Centralstelle and listed as coming from HAGEM. Zygospores formed readily on ordinary media at laboratory temperatures. The (+) race has died, but the (−) is still in cultivation.

MUCOR DISPERSUS Lendner.—This species, provisionally designated as *Mucor* "D" in earlier publications (**12, 13**), has been kindly determined for us by Professor LENDNER. It was obtained in Cold Spring Harbor from fallen leaves which had been sown on sterilized bread. Only a single pair of (+) and (−) races have been studied.

MUCOR MUCEDO Linnaeus.—The term *Mucor mucedo* has been applied probably to a number of distinct species among the unbranched forms with large sporangia. The type we have studied is of common occurrence on horse dung, from which source most of our races have been obtained. Our first (+) and (−) races were isolated in 1903 from a culture which was producing zygospores. By frequent sporangial transfers these (+) and (−) races were kept under cultivation in separate test-tubes for sixteen years. In 1918 the (−) race gradually became weakened in growth, and in 1919 it was impossible to obtain a transfer from the last culture which had reached the 213th sporangial generation. The (+) race died out in 1920, after it had reached the 218th non-sexual generation. It had not shown so much decline in vigor as the (−) race, and its dying out may have been assisted by an infection of mites. This (+) race had always been of greater vigor than its (−) mate, as judged by the fact that high temperatures had less marked effect upon its sexual activity. It has earlier been shown (**2, 3**) that cultivation of this species at temperatures such as are frequent in summer lessens the sexual activity, which may be regained when the races are grown at a low temperature for a few sporangial generations. For this reason, contrasts between the various races tested were made in a cold cellar. The cultures were run on nutrients 391, 360, and on sterilized horse dung.

The thirty-nine races tested represent thirteen gross cultures obtained from nine different localities. As shown in the summary table XII, 86 combinations were made; fourteen races were (+), eleven were (−), and fourteen were neutral. Because of the relatively few combinations made, no detailed table of reactions is given for this species. In addition to the races obtained from nature, we have included in the summary table XII the races of this species secured from zygospore germinations (3). The germ tube and spores in the germ sporangium from a zygospore of *M. mucedo* have been shown to be all of the same sex, either (+) or (−). The growths from 53 zygospores were sexed by contrasts between mycelia obtained from germ tubes or from streaks of the spores in their germ sporangia and the test (+) and (−) races. Of these, twenty-two were (+) and thirty-one were (−). In addition, the spores from germ sporangia from four other zygospores (one + and three −) were plated out and the resulting mycelia sexed. A total of 401 races from these monosporic cultures were (−) and twenty-four (+), but these figures obviously give no indication of the proportion of (+) and (−) germinations. The growths from thirty-four spores from the (+) sporangia were temporarily neutral and are included in the neutral column. By an error in making up the summary table in an earlier paper (5), the (−) and neutral races were unfortunately transposed.

In making tests of growths from zygospore germinations, two or more cultures were grown in a petri dish between the old (+) and (−) testers. In consequence each race functioned as a tester against one or more other races, in addition to the original (+) and (−) strains.

MUCOR N.—This undescribed species, which probably represents a new genus, was discovered by THAXTER on a culture of Brazil nuts in the Harvard laboratory. Its (+) and (−) races have been kept under cultivation for over twenty years. Zygospores have been obtained on nutrient 362 at about 27° C., as well as on other nutrients at laboratory temperature.

MUCOR III.—Mated races of this undetermined species of the racemose type came from a rat dung culture in the Harvard laboratory. The original (+) and (−) races have been kept under cultivation for over twenty years. In addition one (−) race was secured

from horse dung in Cold Spring Harbor and a (+) race each from Brazil nuts purchased in Hickory, North Carolina, and from a soil culture from Cold Spring Harbor. Four combinations were made. Neutrals in this species were not identified for the reason given under *Mucor* IV. Cultures were run on nutrient *362* at about *25°* C.

MUCOR IV.—Of *Mucor* IV, an undetermined species of the racemose type, races 921 and 922 originally came from Cold Spring Harbor in 1902, and the (+) and (−) strains are still in cultivation. In

TABLE VIII

MUCOR IV

GRADE	(+) TESTERS	(−) RACES													
		577	878	922	930	857	871	874	875	881	41	880	876	879
2.54....	921	B	C	B	B	C	C	B	A	C	B	B	C	D
1.23....	855	C	B	C	C	C	C	D	O	C	O	O	O	O
Grade..	.....	2.50	2.50	2.50	2.50	2.00	2.00	2.00	2.00	2.00	1.50	1.50	1.00	0.50

GRADE	(−) TESTERS	(+) RACES					
		921	873	855	872	877	882
2.33....	922	B	C	C	B	B	D
1.00....	857	C	C	C	O	O	O
Grade..	.....	2.50	2.00	2.00	1.50	1.50	0.50

addition, 577, 855, 872–875, 878–882 were from soil collections from the vicinity of Cold Spring Harbor; 857 was from horse dung, 871 from undetermined dung from woods, 876 from sheep dung, and 877 from mouse dung, all from the neighborhood of Cold Spring Harbor. No. 41 was isolated from an infection of corn meal in Storrs, Connecticut. As shown in table VIII, thirteen (−) and six (+) races were contrasted with two (+) and two (−) testers each. On account of the difficulty of being sure of the identification of species in the racemose group, only those races which showed zygospores with at least one of the testers were used in this table; in consequence, no neutral races are listed. Cultures were run on nutrient 362 at a temperature of about *27°* C.

MUCOR V.—*Mucor* V is a form found in 1904 on a culture of horse dung from North Carolina, and its (+) and (−) races have been kept in cultivation ever since. It is perhaps not specifically distinct from *M. hiemalis*, since it has formed zygospores, although in no great abundance, with the strains of this name obtained from the Centralstelle. It differs, however, rather markedly from the Centralstelle material in color. Originally its sexual vigor was extremely high and was therefore used as a tester in imperfect reactions with other species. At the present time it has considerably weakened in sexual activity. Two (+) races were obtained from a culture of Brazil nuts purchased in New York City, and a (−) race from the inside of a stump at Cold Spring Harbor. In all, ten combinations were made with the five races, of which three were (+) and two (−). The contrasts gave C and D reactions. No neutrals are recorded for the reason given under *Mucor* IV. Cultures were run at laboratory temperatures.

MUCOR VI.—The (+) and (−) races of this undetermined *Mucor* were isolated in 1904 from a horse dung culture in the Harvard laboratory. It formed zygospores on the ordinary media, but both races have died out.

MUCOR VII.—The (+) and (−) races of this undetermined species were isolated from a culture of guinea pig dung. Three other races came from a culture on deer dung from Storrs, Connecticut, and one from a soil culture at Cold Spring Harbor. Five combinations were made with the six races, of which one was (+) and five (−). The contrasts give three C and two D reactions. No neutrals were recorded for the reason given under *Mucor* IV. The cultures were run at about 25° C.

MUCOR VIII.—The (+) and (−) races of this undetermined species were isolated from zygosporic material found by THAXTER on cultivated plants of *Hesperis* in Cambridge, Massachusetts. Zygospores have been produced on nutrient 362 at a temperature around 27° C. The (−) race has died out within the last few years.

PARASITELLA SIMPLEX Bainier.—The sexual reactions of this parasitic *Mucor* have been discussed in an earlier paper (**14**). Race P I was obtained from heron dung in Cold Spring Harbor, P II from horse dung in Cold Spring Harbor, P III from horse dung from

Woodbury, Long Island, and P IV from horse dung from Long Beach. It has not been possible as yet to obtain a true imperfect hybridization between the sexual races of *Parasitella* and known (+) and (−) races of other mucors. From their biochemical behavior (**14**), however, we may provisionally assign the term (+) to race P IV and (−) to the other three races. The six possible combinations were made between the four races, of which one is listed as (+) and three as (−). The contrasts gave 1 A, 1 B, and 1 C reaction. The contrast series was run on *Mucor rouxii* as host, which was grown on nutrient 391 at laboratory temperature. Zygospore formation was found to be equally abundant when the cultures of *Parasitella* were grown saprophytically (**14**).

PHYCOMYCES BLAKESLEEANUS Burgeff.—This is the common laboratory *Phycomyces*, a not infrequent growth on dung and universally cultivated under the name of *P. nitens*. BURGEFF (**10**), however, has recently shown that the original *P. nitens* is a distinct species growing spontaneously on fatty material.

Our first (+) and (−) races were obtained in 1903 from rabbit dung from Cold Spring Harbor and North Carolina respectively. The (+) race is still in cultivation, having reached the 270th sporangial generation. The (−) race died out in 1923, however, when it had reached the 259th generation. Thirteen races were obtained from dung, chiefly of rabbit and horse in the neighborhood of Cold Spring Harbor. All the possible 105 combinations were made with the races, of which eleven were (+), one was (−), and three were neutral. The cultures were run on nutrient 360 at laboratory temperature.

In addition to these races obtained from nature, we have included, in the summary table XII, the races of this species obtained from isolation cultures from germ sporangia (**3**). They were tested in the same manner as were the races from zygospore germinations of *Mucor mucedo*. The segregation of sex in germ sporangia of *Phycomyces* is frequently incomplete, and spores are formed which give rise temporarily to bisexual or homothallic mycelia. These homothallic mycelia are not constant races, since they ultimately produce pure (+) and (−) spores in their sporangia. These temporarily bisexual mycelia (**3**) are characterized by the production of curiously contorted

yellowish outgrowths, and are properly considered by BURGEFF (8), from his grafting experiments, to be mixomycelia, or mycelia containing a mixture of (+) and (−) protoplasm. Such homothallic cultures, for convenience, are listed in table XII under the neutral column without meaning that such cultures are devoid of sex. No true neutrals have been found from this series of zygospore germinations, although a certain tendency toward temporary neutrality has been observed. A considerable increase in the number of races listed would occur if we had included streak cultures from germ sporangia and mycelia directly from germ tubes, as well as unpublished data on zygospore germinations in 1913.

RHIZOPUS NIGRICANS Ehrenberg.—The source of the races of *Rhizopus* which have been tested in the present study is given in table IX or in table X, where reference is made by T number to our previous paper (7).

Table IX shows tests with *Rhizopus nigricans*. Twenty races were used as testers, including ten (+) and ten (−) races, and in all 1574 contrast combinations were made with the total 97 races. Of these forty were (+), seventeen were (−), and forty failed to show reactions and were provisionally classified as neutral. This neutrality may have been due in part to the fact that species other than *R. nigricans* were probably included among the races tested (cf. table X).

The zygospores of *Rhizopus* have twice been brought to germination by one of us, once in 1906 and again 1913. On the first occasion the germ tubes produced small sporangia, the spores from which gave rise to weak mycelia which ultimately died. On the second occasion only germ tubes were formed which could be induced to produce neither sporangia nor mycelia. Races of *Rhizopus* have been collected and tested for sexual activity since 1904, and in consequence there is a considerable number of races recorded in the final summary in table XI which were contrasted with our test (+) and (−) races, but which were not used in the special study summarized in table IX.

SYNCEPHALASTRUM RACEMOSUM Cohn.—*Syncephalastrum* is a common infection on Brazil nuts along with *Cunninghamella*. Of the cultures for which a source number is not given, 658 was obtained from a gross culture of sheep dung from Cold Spring Harbor; 659

was from the Centralstelle and was listed as coming from ATKINSON; 660 to 663 were furnished by THAXTER and were derived from Florida, Porto Rico, South Carolina, and California respectively; 664 and 665 originated from Florida and Porto Rico respectively and are the races which have been kept under cultivation for about

TABLE IX

RHIZOPUS NIGRICANS

SOURCE "T" NOS.	GRADE	(+) RACES	(−) TESTERS									
			309	15B	106B	900	675	615	25B	305	674	303
—	3.70	899	A	A	A	A	A	C	A	A	B	A
—	3.20	15A	C	A	A	B	B	D	B	A	A	A
111	3.00	670	B	A	A	B	A	A	A	C	C	O
—	2.90	106A	A	A	A	O	B	A	C	A	O	A
100	2.90	410	B	A	D	B	C	B	B	B	A	B
—	2.60	304	C	B	C	B	A	B	C	C	B	C
—	2.60	314	A	C	C	B	A	B	B	C	B	O
80	2.60	343	B	A	D	A	C	B	D	C	A	C
81	2.50	344	C	B	C	C	A	B	C	A	O	C
—	2.40	306	C	A	A	A	O	B	A	B	O	O
27	2.40	323	O	A	B	B	O	A	A	O	A	C
—	2.20	25A	A	B	B	D	B	B	B	O	C	O
118	2.20	806	B	B	A	B	A	B	C	O	O	O
—	2.00	308	B	D	B	B	B	B	C	O	C	O
—	1.90	177	C	D	A	A	A	O	C	C	O	O
—	1.90	302	O	A	A	B	O	O	A	O	O	A
53	1.80	331	B	C	A	C	C	B	C	O	O	O
—	1.70	175	B	D	O	B	B	C	B	O	C	O
—	1.70	178	C	D	B	A	B	B	O	D	O	O
—	1.60	43	C	D	B	B	B	C	D	D	O	O
—	1.60	170	B	D	B	B	O	B	B	O	O	O
—	1.50	173	C	C	B	B	O	B	C	O	O	O
—	1.50	176	B	D	C	B	O	B	B	O	O	O
27	1.40	325	A	D	C	C	A	O	O	O	D	O
—	1.30	174	C	D	B	C	B	O	D	D	O	O
73	1.30	338	C	D	A	O	B	B	O	O	O	O
—	1.00	313	C	D	C	C	B	O	O	O	O	O
54	0.60	332	O	B	C	D	O	O	O	O	O	O
101	0.60	411	C	A	O	O	O	O	O	O	O	O
—	0.60	601	C	C	O	D	O	O	D	O	O	O
—	0.50	317	C	C	D	O	O	O	O	O	O	O
—	0.40	315	D	D	O	C	O	O	O	O	O	O
13	0.40	322	C	D	O	O	O	D	O	O	O	O
48	0.40	329	D	D	O	C	O	O	O	O	O	O
50	0.30	330	C	D	O	O	O	O	O	O	O	O
—	0.30	600	C	D	O	O	O	O	O	O	O	O
114	0.30	673	O	O	B	O	O	O	O	O	O	O
—	0.20	316	D	D	O	O	O	O	O	O	O	O
39	0.20	327	D	D	O	O	O	O	O	O	O	O
27	0.10	324	O	O	O	D	O	O	O	O	O	O
Grade			2.12	2.07	2.07	2.02	1.70	1.62	1.52	0.87	0.85	0.70

TABLE IX—*Continued*

Source "T" nos.	Grade	(−) races	(+) testers									
			106A	899	15A	670	304	343	25A	806	302	601
—	3.50	15B	A	A	A	A	B	A	B	B	A	C
—	2.90	106B	A	A	B	A	C	D	B	A	A	O
118	2.90	805	B	C	B	A	B	A	B	B	C	C
—	2.70	309	A	A	C	B	C	B	A	B	O	C
118	2.70	675	B	A	B	A	A	C	B	A	O	O
—	2.60	25B	C	A	B	A	C	D	B	C	A	D
—	2.60	900	O	A	A	B	B	A	D	B	B	D
—	2.40	102	A	C	B	O	B	C	B	B	C	C
—	2.30	615	A	C	D	A	B	B	B	B	O	O
76	2.20	337	A	B	A	O	C	D	A	C	O	C
—	2.10	307	A	B	A	C	C	A	O	C	O	O
—	2.00	303	A	A	A	C	C	C	O	O	O	O
—	2.00	305	A	A	A	O	C	C	O	O	A	O
76	1.90	341	B	A	A	B	B	C	O	O	O	O
115	1.80	674	O	B	A	C	B	A	C	O	O	O
—	1.60	616	C	O	O	A	C	D	B	C	O	C
—	1.60	103	C	O	O	A	C	B	C	O	O	B
Grade			3.00	3.00	2.94	2.76	2.53	2.53	2.18	2.00	1.35	1.00

Source "T" nos.	Neutral races	Source "T" nos.	Neutral races	Source "T" nos.	Neutral races	Source "T" nos.	Neutral races
—	73	12	321	96	406	—	608
—	74	38	326	97	407	—	609
—	89	40	328	98	408	—	610
—	101	55	333	99	409	—	611
—	310	60	334	—	602	86	612
—	311	66	335	—	603	96	613
—	312	67	336	—	604	—	614
—	318	74	339	—	605	—	617
—	319	75	340	—	606	112	671
12	320	79	342	—	607	113	672

twenty years as the (+) and (−) representatives of *Syncephalastrum*. The zygospores are small, resembling those in *Mucor*.

Table XI shows the tests with *Syncephalastrum*. Eighteen races were used as testers, including eight (+), eight (−), and two neutral races, and in all 1269 contrast combinations were made with the total eighty races. Of these, thirty-seven were (+), thirty-nine were (−), and four were neutral. The neutrals 281 (T13) and 288 (T48), although contrasted with all the other races, are not listed with the other testers in the table. The zero reactions are also omitted between the (+) and (−) testers and the four neutrals 281, 288, 689

(T111), and 692 (T112). It will be observed that the (+) race 280 formed zygospores in only one of the contrast combinations. In consequence, if the (−) race 195 had not been used as a tester, race 280 would have been listed as a neutral. If the four races provisionally classed as neutral had been contrasted with other stronger testers, it is possible that they would all have given a (+) or a (−) reaction. Cultures were run at a temperature of about 27° C.

TABLE X

RHIZOPUS NIGRICANS

LIST OF RACES SHOWN IN TABLE IX FOR WHICH A SOURCE NUMBER IS NOT THERE GIVEN

RACE NO.	SUBSTRATUM	LOCALITY REPRESENTED	RACE NO.	SUBSTRATUM	LOCALITY REPRESENTED
15A & B	Bread	Durham, N.C.	312	Strawberries	Georgia
25A & B	.....	Amherst, Mass.	313	Black raspberries	Connecticut
43	Boletus	Woods Hole, Mass.	314	Black raspberries	Connecticut
73	Soil	New Brunswick, N.J.	315	Cherries	Washington, D.C.
74	Soil	New Brunswick, N.J.	316	Dewberry	Boston, Mass.
89	Soil	New Brunswick, N.J.	317	.....	Arkansas
101	.....	Panama	318	.....	Arkansas
102	.....	Panama	319	.....	Arkansas
103	.....	Panama	600	Almond nuts	Cambridge, Mass.
106A & B	Bread	Philadelphia, Pa.	601	Lab. culture	Cambridge, Mass.
170	Dung and leaves	Southern China	602	Bread	Toronto, Canada
173	Brazil nuts	Huntington, N.Y.	603	Pistachio nuts	Catania, Italy
174	Almond nuts	Huntington, N.Y.	604	Rhiz. oryzae	Centralstelle
175	Brazil nuts	Huntington, N.Y.	605	Rhiz. oryzae	Centralstelle
176	Eng. walnuts	Huntington, N.Y.	606	Rhiz. arrhizus	Centralstelle
177	Brazil nuts	Huntington, N.Y.	607	M. norvegicus	Centralstelle
178	Brazil nuts	Huntington, N.Y.	608	R. delamar	Centralstelle
302	Sweet potatoes	Huntington, N.Y.	609	R. tritici	Centralstelle
303	Sweet potatoes	Huntington, N.Y.	610	R. chinensis	Centralstelle
304B	Sweet potatoes	Huntington, N.Y.	611	R. nodosus	Centralstelle
305B	Sweet potatoes	Huntington, N.Y.	614	Brazil nuts	Amsterdam, N.Y.
306C	Sweet potatoes	Oyster Bay, N.Y.	615	Papaw	Cuba
307C	Sweet potatoes	Oyster Bay, N.Y.	616	Artocarpus	Cuba
308	Strawberry	Washington, D.C.	617	Anona	Cuba
309	Strawberry	Washington, D.C.	899	Vegetables	Cambridge, Mass.
310	Strawberry	Washington, D.C.	900	Vegetables	Cambridge, Mass.
311	Peaches	Boston, Mass.			

Summary and discussion

In table XII is given a summary of the heterothallic species tested for intraspecific sexual reactions. The classification is based almost exclusively upon the recorded tests made for the purpose of discovering the possible occurrence of sex intergrades. A considerable number of individual tests, made for other purposes during the last twenty years and more, have not been included in the table. Their inclusion would have greatly increased the number of combinations listed. Thirteen records, however, on eighteen races of the species previously classified, are listed separately. These for the most part are contrasts between races which had died out when the special con-

trast series were started and could not conveniently be included with the latter. Records are available for over 500 contrasts between 203 races of various species not otherwise listed. These were obtained in attempting to match up those races in our collection which were similar in appearance. Of the more than 500 combinations thus

TABLE XI

SYNCEPHALASTRUM RACEMOSUM

SOURCE	GRADE	(−) RACES	(+) TESTERS							
			651	648	194	664	659	663	661	660
T81.....	3.00	653	A	C	C	B	A	A	C	B
T40.....	2.87	287	B	B	C	B	B	B	A	C
T96.....	2.87	655	A	A	C	B	B	B	C	C
T50.....	2.75	291	A	A	B	C	B	C	C	C
T74.....	2.75	301	A	A	B	B	C	C	C	C
—.....	2.75	658	A	C	C	B	B	A	O	A
T116.....	2.75	701	B	B	C	B	B	A	C	C
T115.....	2.75	707	B	C	C	A	B	B	B	C
T80.....	2.62	650	A	A	B	B	C	C	D	C
H2.....	2.50	195	A	A	B	C	C	D	C	C
T50.....	2.50	290	A	B	B	C	B	C	D	C
T74.....	2.50	299	B	C	C	B	A	C	C	C
H2.....	2.25	199	A	A	B	B	D	C	D	O
T51.....	2.25	293	A	B	C	C	C	C	D	C
T52.....	2.25	294	A	B	C	C	B	C	O	C
T81.....	2.25	652	A	A	B	C	C	D	D	D
T81.....	2.25	654	A	B	D	C	C	C	C	C
T118.....	2.25	708	A	B	C	B	D	C	C	D
H2.....	2.12	198	B	B	B	B	C	C	O	D
T13.....	2.12	279	A	B	C	D	C	C	D	C
T66.....	2.12	298	A	B	B	C	C	D	C	O
T55.....	2.12	641	A	B	D	B	C	D	C	D
T80.....	2.12	649	A	A	A	D	D	D	D	D
T112.....	2.12	691	C	B	B	C	C	C	C	D
T117.....	2.12	704	B	B	B	C	C	C	C	O
—.....	2.00	665	B	B	C	C	C	C	D	D
T112.....	1.87	693	C	A	C	C	D	D	C	D
T116.....	1.87	700	B	B	C	D	C	C	C	O
T117.....	1.87	706	A	A	C	B	O	O	C	O
T96.....	1.75	656	B	B	C	B	C	O	O	D
T114.....	1.75	698	C	D	B	C	C	C	C	O
H2.....	1.62	197	A	A	B	C	O	O	O	O
T12.....	1.62	276	A	A	B	C	O	O	O	O
T27.....	1.62	284	B	B	C	C	D	D	O	D
—.....	1.62	662	C	B	B	D	D	D	C	O
H2.....	1.37	200	A	B	C	C	O	O	O	O
T75.....	1.25	643	B	A	C	D	O	O	O	O
T114.....	1.12	699	D	D	D	D	D	C	C	O
T117.....	1.00	705	C	B	O	D	O	O	C	O
Grade.....			3.38	3.17	2.31	2.23	1.82	1.67	1.41	1.15

TABLE XI—*Continued*

Source	Grade	(+) races	(−) testers							
			652	195	653	658	705	287	662	665
T13	3.25	283	A	A	B	A	B	C	B	B
T80	3.25	651	A	A	A	A	C	B	C	B
T98	3.12	657	A	A	B	B	B	B	B	C
T12	3.00	277	A	B	B	A	A	C	C	C
T80	3.00	648	A	A	C	C	B	B	B	B
T113	3.00	697	B	A	B	C	A	C	A	C
T54	2.87	297	A	B	B	B	B	C	B	C
T76	2.87	645	B	B	B	A	A	C	C	D
T113	2.87	696	A	B	B	C	B	C	B	B
T118	2.87	710	A	B	B	C	B	B	B	C
H2	2.75	196	A	B	B	C	A	B	D	C
T13	2.75	282	A	B	B	B	A	C	D	C
T117	2.75	703	A	A	C	C	A	B	B	O
T53	2.62	640	A	B	D	C	A	C	B	C
T118	2.62	709	A	A	B	D	B	C	C	C
T51	2.37	292	B	B	B	B	C	C	C	D
T52	2.37	639	C	C	C	A	B	D	C	B
T79	2.25	646	A	B	C	D	B	C	C	D
H2	2.12	194	B	B	C	C	O	C	B	C
H27	2.12	659	C	C	A	B	O	B	D	C
—	2.12	664	C	C	B	B	D	B	D	C
T39	2.00	286	B	B	C	B	B	D	O	D
T79	2.00	647	B	C	C	B	C	D	C	D
—	2.00	663	D	D	A	A	O	B	D	C
T76	1.87	644	B	D	C	B	C	D	C	D
—	1.75	661	D	C	C	O	C	A	C	D
T113	1.75	694	C	C	B	D	C	C	O	C
—	1.62	660	D	C	B	A	O	C	O	D
T50	1.50	289	A	C	C	C	D	O	D	O
T27	1.25	285	D	C	B	O	D	C	O	D
T74	1.25	300	B	D	C	B	O	O	O	D
T54	1.12	296	C	D	D	B	D	D	O	O
T55	0.87	642	C	C	O	D	C	O	O	O
T111	0.87	690	C	C	D	O	D	D	O	O
T113	0.62	695	D	C	O	O	D	D	O	O
T12	0.37	278	D	D	D	O	O	O	O	O
T13	0.12	280	O	D	O	O	O	O	O	O
Grade			2.81	2.54	2.37	2.24	2.00	1.84	1.57	1.43

made, probably the majority represent intraspecific combinations. Since, however, in many cases we could not be certain, without the formation of zygospores, that all of a given group belonged to a single species, some of the contrasts may represent interspecific combinations, and it has seemed best to omit from table XII the number of combinations made within these groups.

The races listed under zygospore germinations of *Mucor mucedo*

and *Phycomyces* are derived in a different manner from those discussed earlier. Since there is presumably segregation following

TABLE XII

SUMMARY OF INTRASPECIFIC REACTIONS

	LOCATIONS	GROSS CULTURES	RACES	COMBINATIONS POSSIBLE	TESTERS USED	COMBINATIONS MADE	(+)	NEUTRAL	(−)
Absidia blakesleeana	13	26	40	780	40	780	19	3	18
caerulea	3	12	22	231	22	231	4	5	13
cylindrospora	....	....	....	1	2	1	1	0	1
dubia	10	18	24	276	8	154	4	0	20
glauca	5	5	10	45	10	45	4	0	6
ramosa	....	....	3	3	2	3	2	0	1
repens	3	5	9	36	2	15	8	0	1
sp. (whorled)	11	15	34	561	34	528	14	2	18
Blakeslea trispora	....	....	2	1	2	1	1	0	1
Chaetocladium brefeldii	1	10	13	78	13	78	?	0	?
Choanephora sp. A	1	1	2	1	2	1	1	0	1
Choanephora cucurbitarum	2	19	33	528	10	275	5	0	28
Circinella spinosa	13	28	55	1485	4	206	34	7	14
Circinella umbellata	2	2	2	1	2	1	1	0	1
Cunninghamella sp. A	9	18	53	1378	6	297	22	2	29
bertholletiae	18	36	89	3916	15	1215	12	8	69
echinulata	7	10	18	153	18	153	10	0	8
elegans	1	16	42	861	12	426	25	1	16
Helicostylum piriforme	4	9	9	36	9	36	6	0	3
Mucor griseo-cyanus	5	8	9	36	2	10	3	0	6
hiemalis	....	....	2	1	2	1	1	0	1
dispersus	2	2	2	1	2	1	1	0	1
mucedo	9	13	39	741	4	86	14	14	11
sp. N	1	1	2	1	2	1	1	0	1
sp. III	3	4	5	10	2	4	3	0	2
sp. IV	5	13	19	171	4	76	6	0	13
sp. V	3	3	5	10	2	5	3	0	2
sp. VI	1	1	2	1	2	1	1	0	1
sp. VII	3	3	6	15	2	5	1	0	5
sp. VIII	1	1	2	1	2	1	1	0	1
Parasitella simplex	3	4	4	6	4	6	1	0	3
Phycomyces blakesleeanus	....	....	15	105	15	105	11	3	1
Rhizopus nigricans	....	....	236	27,730	20	1574	89	85	62
Syncephalastrum racemosum	18	35	80	3160	18	1269	37	4	39
Other races of above species	....	....	18	.....	....	13	7	2	9
Races of species not listed	....	....	203	.....	....	....	40	99	64
Totals	....	....	1111	.....	296	7604	393	235	470
ZYGOSPORE GERMINATIONS									
Mucor mucedo	....	....	512	.....	514	1280	46	34	432
Physomyces blakesleeanus	....	....	392	.....	394	980	258	16*	118
Totals	....	....	904	.....	908	2260	304	50	550
Grand totals	....	....	2015	.....	1204	9864	697	285	1020

* Mixomycelia, not true neutrals.

sexual reproduction, an opportunity would be given among these races for the occurrence of sex intergrades which might not be able to survive in nature, which is the source of our other cultures. All the species in table XII (except the parasitic forms) have been tested by the imperfect hybridization reaction which will be discussed in a succeeding paper in this journal. The parasitic forms, *Chaetocladium* and *Parasitella,* failed to give a true imperfect hybridization reaction with the races with which they were contrasted. On account of their reaction to biochemical tests, it has been possible to assign provisionally the terms (+) and (−) to the opposite sexes of *Parasitella*. *Chaetocladium*, however, has not yet been tested biochemically, and it has not yet been possible, therefore, to list its sexual races in the (+) and (−) columns in the table.

Various grades of sexual vigor are apparent in the different races tested within a single species. While in general there is a more or less orderly decrease in strength of reaction as one goes from contrasts between strong to those between weak races, still there is evidence from the tables for a certain amount of compatibility and incompatibility between certain races. As has been pointed out before, the races listed as neutral are probably, in many cases at least, (+) and (−) races, so weak sexually that none of the testers of the other sex yet contrasted with them has been able to call forth zygospore formation. Thus, in table IX, if the (−) tester 195 had not been used with the (+) race 280, the latter would have been listed as a neutral.

The investigations reported in the present paper were undertaken with the purpose of discovering whether among the heterothallic Mucorales sex intergrades could be found such as are frequently encountered in higher plants. Records are given of tests with over 2000 races included in thirty-four or more species included in twelve genera. If the tests not listed in table XII are included, between 10,000 and 20,000 intraspecific combinations have been made between heterothallic species, without evidence for sex intergrades. We may conclude, therefore, that so far as our tests have gone, the heterothallic mucors are sexually strictly dimorphic.

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SEXUAL DIMORPHISM IN MUCORALES

II. INTERSPECIFIC REACTIONS

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In the preceding paper in this series (5) were given the results of testing together races belonging to the same species. When two sexually active races of the same species are allowed to grow together under proper environmental conditions, zygospores ordinarily result. In distinction from the perfect reactions in these intraspecific contrasts, imperfect reactions may occur in interspecific contrasts, when the two sexually active races belong to different species. Interspecific reactions have been discussed in earlier publications (1, 2, 3) under the term "imperfect hybridization." The reaction may be indicated merely by the formation of small progametes at the point of contact between the (+) and (−) hyphae of the two species. Sometimes the gametes (gametangia) are delimited from one or both of the conjugative hyphae. Rarely a gamete so formed produces a parthenospore or a zygospore (2, 4). These parthenospores resemble small zygospores in appearance, and perhaps have been confused with them at times by other investigators. A careful investigation, however, will show that a perfect suspensor is present only on one side.

Races differing more or less from one another in growth appearance and even in spore characters have been found within many groups which we have considered single species. Our experience with both intraspecific and interspecific reactions leads us to believe that it is most convenient at least to consider races which form zygospores with each other as belonging to the same species. We have never ourselves found zygospore production between races obviously belonging to different species. BURGEFF, however, has obtained good crosses between mutant races of *Phycomyces* (7), and even between two distinct species of *Phycomyces* (8). Other reports of true hybridization among mucors must be accepted with caution, in view of the ease of confusing parthenospores with true zygospores. In general the production of progametes in intraspecific contrasts goes through

TABLE I

SOURCE OF RACES IN TABLE II

SPECIES	SEX	RACE	SOURCE
Mucor H	+	.....	Paradise nuts, New York City
Mucor dispersus	−	.....	Leaves, Cold Spring Harbor
Mucor griseo-cyanus	+	.....	Lab. culture, Cambridge, Mass.
Mucor griseo-cyanus	−	.....	Centralstelle
Helicostylum piriforme	+	863	Rat dung, Cold Spring Harbor
Helicostylum piriforme	−	861	Brazil nuts, New York City
Choanephora cucurbitarum	+	821	Squash, Cold Spring Harbor
Choanephora cucurbitarum	−	832	Squash, Woodbury, L.I.
Mucor V	+	901	Horse dung, North Carolina
Mucor V	−	902	Horse dung, North Carolina
Rhizopus nigricans	+	899	Potato, Cambridge, Mass.
Rhizopus nigricans	−	900	Potato, Cambridge, Mass.
Circinella umbellata	+	897	Rat dung, China
Circinella umbellata	−	898	Dog dung, Cambridge, Mass.
Cunninghamella bertholletiae	+	217	Brazil nuts, Oyster Bay
Cunninghamella bertholletiae	−	266	Paradise nuts, New York City
Choanephora A	+	.....	Raleigh, North Carolina
Choanephora A	−	.....	Raleigh, North Carolina
Absidia glauca	+	915	Centralstelle
Absidia glauca	−	666	Soil, Cold Spring Harbor
Syncephalastrum racemosum	+	651	Brazil nuts, New York City
Syncephalastrum racemosum	−	652	Brazil nuts, Brooklyn, N.Y.
Cunninghamella elegans	+	496	Soil, Cold Spring Harbor
Cunninghamella elegans	−	506	Soil, Cold Spring Harbor
Mucor N	+	.....	Cambridge, Mass.
Mucor N	−	.....	Cambridge, Mass.
Mucor IV	+	921	Cold Spring Harbor
Mucor IV	−	922	Cold Spring Harbor
Absidia blakesleeana	+	369	Brazil nuts, Amsterdam, N.Y.
Absidia blakesleeana	−	571	Brazil nuts, Worcester, Mass.
Mucor III	+	.....	Rat dung, Cambridge, Mass.
Mucor III	−	.....	Rat dung, Cambridge, Mass.
Mucor VII	+	.....	Guinea-pig dung
Mucor VII	−	.....	Guinea-pig dung
Circinella spinosa	+	426	Brazil nuts, Worcester, Mass.
Circinella spinosa	−	413	Brazil nuts, Huntington, L.I.
Mucor VIII	+	.....	Hesperis, Cambridge, Mass.
Mucor VIII	−	.....	Hesperis, Cambridge, Mass.
Absidia caerulea	+	533	Soil, Cold Spring Harbor
Absidia caerulea	−	912	Rabbit dung, Mt. Katahdin, Me.
Cunninghamella echinulata	+	885	Lab. culture, Cambridge, Mass.
Cunninghamella echinulata	−	886	Lab. culture, Cambridge, Mass.
Absidia cylindrospora	+	891	Centralstelle
Absidia cylindrospora	−	892	Centralstelle
Absidia repens	+	895	Brazil nuts
Absidia repens	−	896	Nuts, Margarita Island, Venezuela
Absidia whorled	+	395	Brazil nuts, Brooklyn, N.Y.
Absidia whorled	−	400	Brazil nuts, Storrs, Conn.
Phycomyces blakesleeanus	+	893	Rabbit dung, Cold Spring Harbor
Phycomyces blakesleeanus	−	894	Rabbit dung, North Carolina
Mucor VI	+	.....	Horse dung, Cambridge, Mass.
Mucor VI	−	.....	Horse dung, Cambridge, Mass.
Mucor mucedo	+	.....	Horse dung, North Carolina
Mucor mucedo	--	.....	Horse dung, North Carolina

to the formation of zygospores. Sometimes, however, as in certain contrasts of races of *Rhizopus*, many of the conjugations abort in various stages of development, leaving only a few perfect sexual spores. In *Cunninghamella bertholletiae* (4) certain combinations constantly gave only early stages of conjugation. Why this is so is not known, although in a few cases it was shown that perfect zygospore production could be induced in these cases by making the inoculations closer together.

The classification of races of mucors according to their sex by means of the imperfect hybridization reaction has been a common procedure in our laboratory. It seemed desirable, however, to study the reactions in more detail by making all possible combinations between selected mated pairs of races of the heterothallic species available.

The tests summarized in table II were made in 1919 and 1920, except those with *Muco mucedo* and *Mucor* VI, which are from earlier records. Races of these two species had become weakened or had died out at the time the series was tested. A few others of the species discussed in our preceding paper on intraspecific reactions are not included in the present study for similar reasons, or because the mated races were not available when the tests were made. Table I lists, with sources, the races according to the order in which they appear in table II. As may be seen by checking their numbers with the tabulations in our preceding paper, an attempt was usually made to use, in the interspecific tests, the strongest (+) and (−) races from each species as judged by their ability to take part in zygospore formation. *Mucor* H and *M. dispersus* are unmated (+) and (−) races which had been found especially valuable as imperfect hybridization testers. Since these experiments have been completed, the mate to *M. dispersus* has been found. Except for these two races, *Mucor* H and *M. dispersus*, the races of a given species are mated, that is, have produced zygospores when contrasted together.

The technique in making contrasts has already been described (6). The races to be contrasted were grown in pairs in watch glasses on nutrient 362 (2 per cent agar, 2 per cent whey powder, and 1 per cent dextrose), which seems to be the best medium for imperfect reactions of those tested, possibly in part because it does not support a too luxurious growth of aerial hyphae. Cultures were run in an oven

at a temperature around 24° C. *Mucor mucedo* and *Mucor* VI had been tested earlier under other conditions.

Since some species grow faster than others, there were four main groups established which were planted at 8 to 12 hour intervals. In the first group were the slowest growers, and these were planted

TABLE II

INTERSPECIFIC SEXUAL REACTIONS

(+) RACES	(−) RACES: Mucor H	Mucor griseo-cyanus	Helicostylum piriforme	Choanephora cucurbitarum	Mucor V	Rhizopus nigricans	Circinella umbellata	Cunninghamella bertholletiae	Choanephora A	Absidia glauca	Syncephalastrum racemosum	Cunninghamella elegans	Mucor N	Mucor IV	Absidia blakesleeana	Mucor III	Mucor VII	Circinella spinosa	Mucor VIII	Absidia caerulea	Cunninghamella echinulata	Absidia cylindrospora	Absidia repens	Absidia whorled	Phycomyces blakesleeanus	AVERAGES	Mucor VI	Mucor mucedo
Mucor dispersus	A	B	B	B	C	B	O	C	C	D	C	C	C	C	B	D	C	C	O	C	C	C	O	O	O	1.80	..	..
Mucor griseo-cyanus	A	Z	A	A	O	C	C	C	B	O	B	C	B	C	O	C	O	O	D	O	C	C	O	O	O	1.58	..	..
Helicostylum piriforme	A	A	Z	A	C	A	B	A	A	D	C	A	C	B	C	C	B	B	B	C	O	D	O	O	O	2.38	..	..
Choanephora cucurbitarum	A	B	A	Z	D	C	B	B	B	D	D	O	C	B	D	B	B	B	O	O	C	C	O	O	O	1.83	..	..
Mucor V	A	A	C	B	Z	B	C	C	A	C	C	C	B	C	D	B	B	C	C	C	C	D	O	C	O	2.21	H	H
Rhizopus nigricans	A	B	B	C	D	Z	O	C	C	O	A	C	D	O	B	O	C	D	O	C	O	O	O	D	O	1.38	H	..
Circinella umbellata	A	B	C	B	O	O	Z	C	D	C	O	C	A	C	C	A	O	B	C	C	O	O	O	O	O	1.58	..	..
Cunninghamella bertholletiae	A	C	O	C	A	B	O	Z	C	B	C	C	C	C	O	O	C	O	C	O	C	D	O	O	O	1.46	..	..
Choanephora A	C	B	C	C	C	O	A	D	Z	O	O	O	O	C	D	C	C	B	O	C	O	O	O	C	O	1.25	..	..
Absidia glauca	A	A	D	B	C	A	C	A	D	Z	C	C	B	A	O	C	B	O	O	D	C	C	O	O	O	1.92	..	..
Syncephalastrum racemosum	A	B	C	D	C	A	O	B	B	D	Z	B	D	O	O	O	C	O	O	O	O	D	O	O	O	1.25	..	..
Cunninghamella elegans	B	A	O	O	C	C	O	C	O	O	D	Z	C	C	A	D	O	O	C	D	O	O	O	O	O	1.08	.	..
Mucor N	C	B	O	B	O	C	C	C	O	O	D	C	Z	C	O	O	C	D	O	O	O	O	O	O	O	0.92	H	..
Mucor IV	A	A	B	A	O	C	C	O	O	O	D	O	C	Z	O	O	O	O	O	O	O	O	O	O	O	0.92	..	..
Absidia blakesleeana	A	A	B	C	D	C	C	O	B	O	B	B	C	B	Z	O	O	O	O	D	O	O	O	O	O	1.38	..	..
Mucor III	C	A	A	B	O	O	A	D	B	O	O	B	O	O	O	Z	O	O	O	O	O	C	O	C	O	1.17	H	..
Mucor VII	A	B	B	A	O	O	B	O	B	O	O	O	O	O	O	O	Z	O	O	O	O	O	O	O	O	0.83	..	..
Circinella spinosa	A	A	B	C	O	C	C	O	O	O	O	C	C	O	O	O	O	Z	O	O	O	O	O	O	O	0.88	..	..
Mucor VIII	C	C	C	B	B	O	C	C	C	C	O	C	C	C	O	O	O	O	O	Z	O	C	O	O	O	1.08	..	..
Absidia caerulea	B	C	D	C	C	C	O	O	O	C	O	O	O	O	O	O	O	D	O	O	Z	O	C	O	D	0.75	H	..
Cunninghamella echinulata	A	B	D	C	C	C	O	C	C	C	O	C	O	O	O	O	O	O	O	O	O	Z	O	O	O	0.83	..	..
Absidia cylindrospora	B	C	O	C	O	O	O	C	O	D	O	O	O	O	O	O	C	O	O	O	D	O	Z	O	O	0.54	..	..
Absidia repens	B	C	D	O	O	O	C	D	O	C	D	O	B	C	O	O	O	O	O	O	D	O	O	Z	O	0.75	..	..
Absidia whorled	C	O	O	O	C	C	O	O	O	O	D	O	O	O	O	O	O	O	O	C	O	O	O	O	Z	0.38	..	..
Phycomyces blakesleeanus	C	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	Z	0.08	..	H
AVERAGES	3.36	2.88	1.75	2.25	1.16	1.71	1.46	1.54	1.58	0.67	1.25	1.38	1.50	1.29	1.71	0.92	1.04	0.75	0.50	0.79	0.58	0.67	0.00	0.33	0.00		:	:
Mucor VI	..	..	..	..	H	H	..	..	..	..	..	..	H	..	..	O	..	..	..	H	..	..	..	..	..		Z	H
Mucor mucedo	..	..	..	..	H	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	H		H	Z

first. These included *Absidia blakesleeana*, *Circinella spinosa*, *C. umbellata*, *Phycomyces*, and *Mucor dispersus*. Most of the species belong in the second group. *Cunninghamella bertholletiae*, *C. elegans*, and *Mucor* H comprised the third group. *Rhizopus*, *Cunninghamella echinulata*, and *Choanephora cucurbitarum* were in the fourth group, and, being the most rapid growers, were planted last. In cases in which growth was poor, or in which bacterial infection had occurred, the contrasts were repeated for the final record.

The contrasts were examined under the Greenough binocular two or three times at intervals determined by the amount of growth, the contact of the two races, etc. Contrasts, which by their last examination had not shown reactions, were further examined by teasing out bits of the interwoven hyphae of the two species under the compound microscope. By this latter more tedious method some strong and several weak reactions were disclosed which had not been apparent at previous examinations with the binocular, probably because of the occurrence of the reaction in these cases after the line of contact had become overgrown with aerial hyphae. After six to seven days zygospores were found in the control contrasts between (+) and (−) races of the species used as a tester. Parthenospores were formed in the contrast between "Whorled" *Absidia* and *Absidia caerulea*, and were not infrequent in combinations with *Mucor* H.

The strength of the imperfect reactions was graded from A to O, as was done in recording zygospore formation in our preceding paper. In making up table II, the grades of all the reactions with a given race were averaged by making A equal 4, and D equal 1, and the species were arranged according to the average rank of their paired (+) and (−) races. In making up the averages, the reactions (H) with *Mucor mucedo* and *Mucor* VI were not included. With the exception of these two species, all the possible 1225 combinations were made between the 25 pairs of (+) and (−) races. Sixteen contrasts are recorded involving *Mucor mucedo* or *Mucor* VI, making a total of 1241 combinations between races of 28 species included in 9 genera. Contrasts between races of like sex in all cases gave O reactions, and therefore are not recorded in the table. Since, as already pointed out, the grades of zygospore formation in different species are not comparable, perfect reactions between the selected pairs of a given species are not assigned grades in the table, but are indicated merely by the letter Z. Their relative sexual activity in zygospore production in comparison with other races of the same species may be seen from their grades in the preceding paper (5).

Table II shows that there is considerable difference in the strengths of imperfect hybridization. This difference seems to have no close connection with the taxonomic relationship of the species in question. The (+) strain, *Mucor* H, is the strongest of all, reacting with all the selected (−) races of the other species tested with grades

of C or better. Unfortunately no (−) *Mucor* H has yet been found. In general, in spite of a certain amount of irregularity, the races as arranged show the strongest imperfect reactions in the upper left-hand portion of the table, while in the opposite corner below the reactions are weak or zeros. The two members of a pair of races chosen for the test in many cases differ considerably in their ability to react with the selected races of other species. Thus the (−) race of *Absidia blakesleeana* has a grade for imperfect hybridization of 0.71, while its (+) race has a grade of 1.38. The two races have about the same grades for zygospore production (2.55 and 2.53). Likewise the (−) race of *A. glauca* with a grade of 0.65 is weaker than the (+) race of the same species with a grade of 1.92. It is possible that the races strongest in imperfect reactions would be found to average somewhat stronger also in zygospore production. The strength of reaction in the two processes, however, cannot be closely correlated. Thus *Helicostylum* ranks among the strongest species in producing imperfect reactions with other forms, but is one of the weakest in the production of zygospores. *Phycomyces*, on the other hand, is perhaps the most difficult species with which to induce the imperfect reaction, but gives zygospores in great abundance under the proper conditions of growth. External conditions apparently affect the processes differently. Thus it has been found that *Cunninghamella echinulata* will form zygospores only at temperatures above 20° C., but will enter into the imperfect hybridization reaction with other species at lower temperatures.

Rather extensive tests of the intraspecific sexual reaction leading to the formation of zygospores reported in our preceding paper have given no evidence for sex intergrades in the heterothallic races investigated. The present study of interspecific reactions, in which an imperfect reaction called "imperfect hybridization" is brought about, furnishes further evidence that sex intergrades in heterothallic mucors are at least extremely rare in nature, if not non-existent. The absence of sex intergrades in this group, in comparison with their frequency in dioecious flowering plants is perhaps connected with the fact that in the mucors we are considering gametophytes, whereas in the flowering plants we are considering sporophytes.

The establishing of a condition of strict sexual dimorphism in the forms studied, as well as their morphological simplicity of sex differ-

entiation, renders the group peculiarly adapted for use in an investigation of the fundamental differences between sexes. The fact that in heterothallic species all the races of one sex are theoretically capable of giving an imperfect reaction with all the sexually opposite races of every other species, while both sexes have been found to give imperfect sexual reactions with homothallic species (**2**), has led to the belief that there must be something fundamental common to all the (+) races, for example, responsible for these reactions. This belief was at the basis of the investigation now in progress (**9, 10, 11**), in which it has been shown that the (+) races of mucors correspond in biochemical behavior to the females of higher plants and animals, and the (−) to males.

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THE NATURE OF CHROMOSOMES

I. EFFECTS OF REAGENTS ON ROOT TIP SECTIONS OF VICIA FABA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 372

R. O. EARL

(WITH NINETEEN FIGURES)

Introduction

Since SUTTON (30), in 1902, first demonstrated the parallelism between the distribution of the chromosomes at meiosis and the incidence of hereditary characters, there has been a great and steadily increasing interest in the chromosomes. The work of MORGAN (23) and his associates and of many other investigators has demonstrated quite clearly that the chromosome theory of heredity rests upon a secure foundation of fact.

Among the decisive evidences may be mentioned chromosome aberrations, worked out genetically and cytologically, in the case of non-disjunction, by BRIDGES (3, 4), and afterwards developed by him in his studies on sex determination, in which he formulated his gene theory of sex; later induced artificially by MAVOR (21) by means of X-rays, and also brought about by many workers by means of species hybridization. Among these may be noted GOODSPEED and CLAUSEN (9), COLLINS and MANN (7), KIHARA (14), and SAX and GAINES (27). Chromosome aberrations have also occurred frequently in *Datura*, where they have been analyzed extensively by BLAKESLEE and BELLING (2). Other evidences are derived from sex-linked inheritance, the number and size of linkage groups, and the time and place in the life cycle of crossing over (PLOUGH 26).

All this evidence clearly shows that hereditary qualities are associated with particular chromosomes, and that chromosomes once lost are not regenerated, nor are the characters for which they were responsible regained. It goes farther. It places the gene theory, that heredity is due to material particles or genes arranged in linear order

in the chromosomes, on so secure a basis, and supports it with such a volume of quantitative evidence of the most precise nature, that it becomes of great importance that further cytological information about the chromosomes should be obtained.

The visible and readily stainable material, chromatin, of which the chromosomes appear to be mainly composed, at least at certain phases, is considered by the chemists to be relatively simple and comparatively uniform throughout living forms. MATHEWS (**20**) states:

> Chromatin apparently consists always of a salt of nucleic acid with a protein base. The nucleic acid is apparently the same, or at any rate closely similar in all the different cells examined; but the protein base is either a very basic, simple protein belonging to the group of protamins . . . . or it is a histon . . . . or it is a more complex and less basic protein of unknown nature in other nuclei.

One of the properties of chromatin is that it is very easily soluble in dilute sodium phosphate.

The chemical evidence thus appears to be distinctly unfavorable to the gene theory, which postulates a series of very unlike substances in the chromosomes, and not a material of uniform chemical composition. As MATHEWS says, "The foregoing discussion of the composition of chromatin . . . . lends no support to the hypothesis that the chromosomes are made of genes." We must therefore consider the evidence from cytology as to the physical nature of the chromosomes.

At present there are two main theories as to their physical constitution. According to one (GREGOIRE and WYGAERTS **10**, SHARP **28**) the chromosomes owe their different appearances at different stages in their life cycle to varying degrees of alveolation. The alveoli are filled with a non-staining substance, and when alveolation is most pronounced anastomosing strands of the staining substance or chromatin obscure the limits of the individual chromosome. No constant skeletal structure is recognized, and it is difficult to interpret the gene theory in its absence. If, however, the chromatin, which according to this school owes its threadlike appearance to the size and disposition of the alveoli, really has an intrinsic filiform nature of its own, we would still have much the same appearance as the proponents of the alveolation theory report. The significance of such a thread, however, might be very great.

It is this thread or chromonema hypothesis which forms the thesis of the other school. It has a long history, having been sponsored periodically since BARANETZKY (1) in 1880 reported a spiral thread in the chromosomes of several species of *Tradescantia*. It has been revived by the recent researches of MARTENS (18, 19), KAUFMANN (12, 13), KUWADA (15), and SHARP (29). All of these workers see a threadlike peripheral band or bands in the chromosome, and an interior substance which varies in staining capacity, and may interchange material with either the nucleolus or the karyolymph. The threadlike bands, or chromonemata, might conceivably carry the genes; and if they were to split lengthwise at each mitosis, as is maintained by some (notably KAUFMANN), the theory of the gene and this hypothesis would be in harmony, even if, as KAUFMANN maintains, the thread is double at all stages. If the number of threads in a chromosome remains constant, as is in fact claimed, it is immaterial how many there may be.

It is to be noted that MORGAN (22) has computed in three ways the maximum probable size of the gene in *Drosophila*, and has estimated its diameter as not exceeding 77 $\mu\mu$. Since the most minute particle which we may hope to render distinctly visible must have dimensions of at least 0.2 μ, it is apparent that particles of the preceding size are much below the limits of microscopic visibility. The number of genes in this insect has been estimated through the frequency with which the same point mutation occurs. This number has then been divided into the size of the sperm head, the combined size of the metaphase chromosomes, and the total estimated volume of the chromosomes at the synapsis stage. This gives the gene size as 77, 60, and 20 $\mu\mu$ respectively. The disparity here may be due partly to changes of volume due to fixation, but indicates more probably that the sperm nucleus and the metaphase chromosomes contain something besides genes. If this were true also for the synaptic chromosomes, the gene size on the basis of these computations would be further reduced. It is interesting to note that the size of a haemoglobin molecule has been estimated as 2.5 $\mu\mu$. While it is realized that no great reliance should be placed on these estimates of gene size, they are significant as an indication of possibilities in *Drosophila* and doubtless other forms as well.

The chemist considers chromatin to be a relatively simple homogeneous substance, varying little from form to form, and being most similar in animals and plants that are most closely related. Cytologists are agreed that the chromatin between mitoses forms anastomosing strands which grow out and connect with those of other chromosomes. The evidence of LEWIS and LEWIS (17) upon this point appears to be conclusive. KAUFMANN (12) reports a variation in the thickness and density of the chromonemata, and suggests that this is due to an interchange with the material of the nucleolus.

If the chromonema, or the chromatin, as displaced by alveoli, were to consist of an ultramicroscopic chain of genes associated with a varying amount of visible, homogeneous, readily staining material, we should have a situation in accord with the apparent morphology of the chromosomes and with the theory of the gene. It would also satisfy the chemist, who would realize that his analyses were of chromatin only, the genes being too small and too few of a kind to be distinguished by his methods, or to affect his determination of the constitution of chromatin.

Since it is notoriously difficult not only to observe accurately at very high magnification, but also impossible to be sure that material, after being killed, fixed, dehydrated, and stained, is still in its natural space arrangement, additional evidence from other sources is desirable.

Material and methods

As chromatin is readily soluble in dilute sodium phosphate, it was thought that it might prove profitable to observe the effects of this and other reagents upon sections of suitable material. Since *Vicia faba* has frequently been used for cytological research, notably by SHARP (28), and since root tips are readily obtained, this plant was chosen for study.

The comparative effects of various fixing agents were first examined. Chromoacetic-osmic mixtures appeared to produce the most natural effects, giving little or no evidence of distortion, and resulting in sharp staining of the chromatin when haematoxylin was used. Formalin-acetic-alcohol was also good. Absolute alcohol alone proved very unsatisfactory, causing much shrinkage of cell contents, and evident distortion. Carnoy's absolute alcohol-chloroform-acetic acid

formula gave much better results, but the finer chromatin threads often appeared irregularly coagulated and sometimes run together. No attempt was made to follow through the whole chromosome cycle, only critical phases being examined in detail.

It was considered that fixing agents containing compounds of heavy metals would prove unsatisfactory where the effects of reagents on sections were being tested, since stable salts of chromatin with these heavy metals would thus be formed. This was found later to be the case, and to apply also to mixtures containing formalin. Carnoy's fluid was therefore selected at the start, and, although other agents were tried, this proved the most satisfactory, using somewhat more acetic than indicated in the formula.

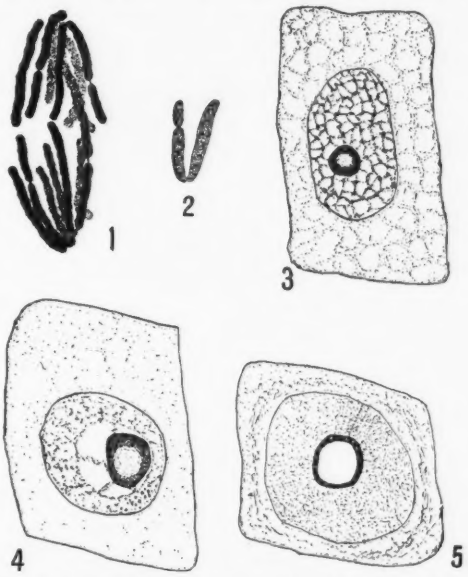
The material was imbedded in paraffin, and sections cut and mounted in the usual way. They were brought through xylol-alcohol and alcohol-water mixtures to water, when some slides were treated in aqueous solution of various reagents, while controls were stained directly. Solutions of Na_3PO_4 from 0.25 to 10 per cent, and similar strengths of NaH_2PO_4 were used. Some were then stained with safranin, a basic stain, some with phloxine, an acid stain, and others with iron-alum haematoxylin and orange G. The short method of mordaunting an hour and staining with haematoxylin an hour, as described by KAUFMANN (**12**), was used with good results.

In order to offset the effects of acidity or alkalinity of the reagent, a 2 per cent solution of NaH_2PO_4 was brought to pH 5 by titration with NaOH, using methyl red as an indicator. This H-ion concentration was selected because it was found by NAYLOR (**25**) to be near the isoelectric points of the materials of the cell. Other reagents also were used, including NaOH of the same alkalinity as 2 per cent Na_3PO_4 as determined by titration with phenolphthalein, and 1 per cent pepsin in 0.1 hydrochloric acid. Further, some slides after treatment with the basic phosphate were placed for some time in 0.1 N acid before staining.

Living root tips were also sectioned on the freezing microtome and the sections treated directly with various reagents. It was considered that cell permeability would be promoted by freezing, and thus the fresh protoplasm would be exposed directly to the reagent. Great difficulties were encountered in handling these sections, however, and the results obtained were inconclusive.

Results

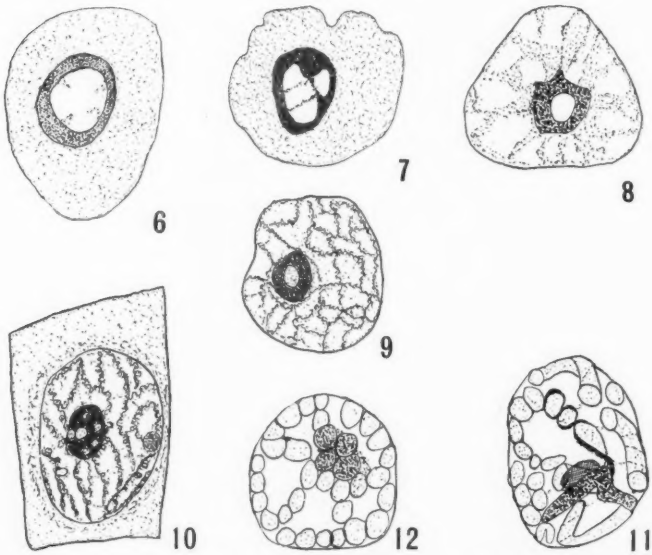
The anaphase condition (fig. 1) in material fixed in chromo-acetic-osmic and stained in iron-alum haematoxylin by the short method shows some evidence of a double chromonema, as pictured by KAUFMANN. Constrictions or achromatic bridges at points of spindle fiber attachment and elsewhere are visible, and also some indication of satellites. With Carnoy's fluid the appearance is very similar (fig. 2). The resting nucleus fixed in chromo-acetic-osmic (fig. 3) shows no positive detail. The nucleolus appears to be a double organ. With Carnoy's (fig. 4) the results are similar, but fixation does not appear to be so good.



FIGS. 1–5*.—Fig. 1, anaphase chromosomes showing achromatic bridges; fixed in chromo-acetic-osmic solution and stained in iron-alum haematoxylin. Fig. 2, anaphase chromosome fixed in Carnoy's fluid and stained in iron-alum haematoxylin. Fig. 3, resting cell, chromo-acetic-osmic; iron-alum haematoxylin. Fig. 4, resting cell, Carnoy; iron-alum haematoxylin. Fig. 5, resting cell, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin.

* All figures were made at table level with the aid of a camera lucida on a Spencer microscope equipped with substage condenser, N.A. 1.40, Leitz 20×Periplan ocular, and Leitz 2 mm. apochromatic objective; magnification 2500.

When the sections are treated with Na_3PO_4 (0.5 per cent or stronger) before staining, no appreciable effects are to be observed if fixation has been in chromo-acetic-osmic or formalin-acetic alcohol;



FIGS. 6–12.—Fig. 6, nucleus only, fixed in absolute alcohol; 1 per cent Na_3PO_4; stained with phloxine. Fig. 7, nucleus only, Carnoy; 2 per cent Na_3PO_4 1 hour then 0.1 N H_2SO_4 one-half hour; stained in haematoxylin and orange G. Fig. 8, early prophase nucleus, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin and orange G. Fig. 9, prophase nucleus a little later than fig. 8 and treated like it. Fig. 10, prophase cell, later stage than fig. 9, same treatment. Fig. 11, prophase nucleus later than fig. 10, same treatment; nucleolus breaking down and appears to be flowing along spiral thread in chromosome; some chromosomes already formed where solution appears complete. Fig. 12, similar stage to fig. 11.

but with absolute alcohol or Carnoy's fluid as fixing agents there is a pronounced effect. The nucleus and chromosomes swell and lose their staining capacity, whether for acid or basic stains. The only exception to be noted is the nucleolus, where the peripheral region takes up the stain. With haematoxylin and orange G it colors light brown, with safranin and with phloxine, red.

In the so-called resting stage the region between the nucleolus

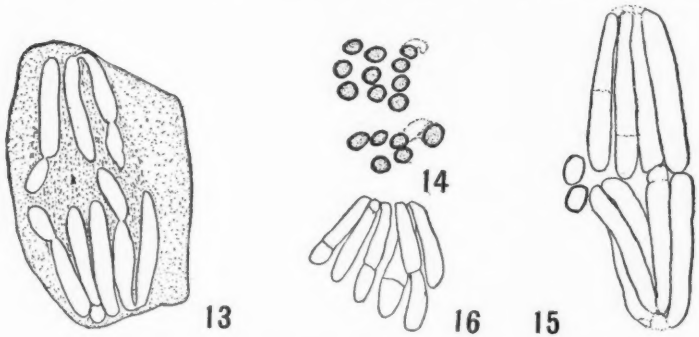
and the nuclear membrane has usually a radiate appearance, due to the process of swelling that has occurred (fig. 5). The cytoplasm is visibly crumpled by the expansion of the nucleus, often much more than is indicated in the drawing. Fig. 6 is a similar drawing of a nucleus only, but stained in phloxine instead of haematoxylin. The appearance of duality in the nucleolus is here pronounced. Fig. 7 is of a nucleus treated with 0.1 N H_2SO_4 after Na_3PO_4.

In early prophases evidence of some organization appears, but distortion is too great to permit of any interpretation (fig. 8). Later, however, reticulate units are seen (fig. 9) which appear to consist essentially of spirally twisted threads. In fig. 10 there is some evidence of a split in these threads, but the material stains so poorly that there could be no assurance on this point. The thread does not appear to be continuous. A still later stage (fig. 11) shows the organization of chromosomes in progress. Some of these appear to have taken definite shape, and resemble somewhat twisted cylinders with evidence of spiral markings. Others appear at first as an orderly arrangement of globules, but on closer examination, especially at some points, resemble more a cylindrical mass with spiral constrictions. It is as if a thread or threads were wound about this mass, which had swollen out through the meshes. The nucleolus, here stained brown due to haematoxylin and orange G, is partly disorganized, and appears to be flowing on to these threads and then throughout the chromosomes. Fig. 12 shows a less evident dispersal of the nucleolar matter, and an arrangement of the globular matter such that the spiral effect is not so pronounced.

Fig. 13 shows anaphase chromosomes. No indication of internal structure is visible, but the chromosome itself appears to be a homogeneous jelly-like mass inclosed in a definite skin or peripheral layer. Constrictions are still visible. Fig. 14, showing cross-sections of anaphase chromosomes, indicates this clearly, as do also the cut ends of two chromosomes in fig. 15. It is to be recalled that such structure was found by CHAMBERS (**6**) in his microdissection studies of chromosomes in living cells. That acidity does not affect the staining reaction is shown by figs. 14 and 16. These preparations are of anaphase chromosomes treated for one hour in Na_3PO_4, and afterwards with 0.1 N acid for half an hour before staining.

The telophase shows a mass of swollen chromosomes very tightly packed within a membrane. As in the anaphase condition, no internal chromosome structure is visible (fig. 17).

With pepsin hydrochloric acid no effect on the nucleus or chromosomes was observed. This is in accord with the work of ZACHARIAS (34), who treated living epidermal tissue and spermatozoa, and of JÖRGENSSEN (11), who used sections of alcohol-fixed animal egg. The sections came off the slide at first, owing to digestion of the egg

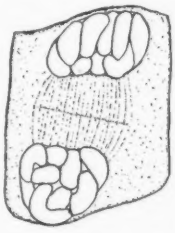


FIGS. 13–16.—Fig. 13, anaphase chromosomes, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin; chromatic material disappeared. Fig. 14, anaphase chromosomes in cross-section, Carnoy; 1 per cent Na_3PO_4 then 0.1 N HCl; iron-alum haematoxylin and orange G; peripheral layer of chromosomes apparent. Fig. 15, anaphase chromosomes, fixed in absolute alcohol; 1 per cent Na_3PO_4; phloxine. Fig. 16, anaphase chromosomes, Carnoy; 2 per cent Na_3PO_4 then 0.1 N H_2SO_4; iron-alum haematoxylin and orange G.

albumen. This was corrected by using a weak solution of potassium bichromate for floating out the paraffin ribbon on the slide, and then exposing the slides to light. The only apparent effect of the pepsin was the partial or complete digestion of the achromatic figure and the rendering more clear of the resting nucleus. This suggests that this figure may have some relation to the karyolymph, since both seem to be digested by the same enzyme. The chromosomes here stand out very clearly, especially in the metaphase. Fig. 18 shows part of an equatorial plate, where satellites and achromatic bridges will be observed, as well as the twisting about each other of the split halves, particularly noticeable in one case. This twisting is very pro-

nounced in earlier stages, and raises a query as to its significance that will be considered later.

A telophasic nucleus in pepsin-treated material is shown in fig. 19. Here the chromosomes stand out quite clearly, giving the impression of a cylindrical mass with a definite surface layer, within which lie two spirally intertwined chromatic threads. Connecting the loops and neighboring chromosome arms run anastomosing strands. Of course these chromosomes might be interpreted as vacuolate, but the impression one gets in focussing up and down is of spiral threads.



FIGS. 17–19.—Fig. 17, telophase, Carnoy; 1 per cent Na_3PO_4; iron-alum haematoxylin and orange G. Fig. 18, metaphase chromosomes, Carnoy; 1 per cent pepsin in 0.1 N HCl 2 hours; iron-alum haematoxylin and orange G. Fig. 19, telophase nucleus treated as for fig. 18.

With NaH_2PO_4 brought to pH_5 by titrating with NaOH, no appreciable effect was observed, except that the nucleolus stained a deeper black with haematoxylin than in untreated sections. The same result was obtained with NaH_2PO_4 alone in various concentrations from 1 to 10 per cent.

With NaOH of the same alkalinity as 2 per cent Na_3PO_4, results were obtained comparable with the effect of Na_3PO_4, but in less degree. The effects observed with the latter reagent are therefore not considered to be due to its alkalinity alone. MATHEWS (**20**) notes that chromatin is more soluble in dilute sodium phosphate than in sodium hydroxide.

Discussion

While the exposure of nucleus and chromosomes to reagents cannot be expected to give a natural picture of their constitution, yet

differential reactions of their constituents to these reagents would expose these differences which otherwise might not be apparent, and might throw some light on such problems as the genetic continuity of the chromosomes or of part of them, and the homogeneity or lack of it in the chromosomes themselves.

The resting nucleus when treated as described shows no sign of any regular structure whatsoever, but in the prophases an orderly arrangement comes into view. It is true that this arrangement may be variously interpreted. In terms of the chromonema hypothesis the constrictions visible in figs. 11 and 12 are due to the crossing of the dual threads, and this is more apparent in the preparations than can be drawn in two dimensions. But this involves two problems. A single row of these beadlike substances corresponds apparently to one chromosome. There is no evidence of a longitudinal split. Can it be that these two threads would later disentangle themselves and form a chromosome each? At present they appear to be definitely inclosed within the membrane of one chromosome. Or is each row only a split half of a chromosome? If so, the threads appear to be much farther separated than would be expected at this stage, on the basis of KAUFMANN'S theory. Of course the swelling caused by the sodium phosphate might be expected to intensify the separation somewhat. I have not been able as yet to elucidate these matters.

The anaphase appearance might be considered due to the solution from the achromatic core of the chromatic element which is based upon the chromonemata; but if it dissolves entirely in the anaphase and telophase, why should the chromonema be visible in the prophase? This might be due to the circumstance that in the anaphase and preceding stages the chromonema is probably reinforced by and reacts with material from the nucleolus to form a substance soluble in sodium phosphate. Before this union both are insoluble in this reagent, and both appear in treated prophases. This view is reinforced by the findings of VAN CAMP (31), who worked with combinations of stains on nuclear material. He considers that the chromatic reticulum and the nucleolus form a special complex, "kinochromatin."

The nucleolus seems clearly to consist of two substances. This is evident in normal preparations and has long been recognized. It was

described by CHAMBERLAIN (**5**) in 1899, and is dealt with in detail in the most recent paper on the subject (LATTER **16,** 1926).

There is nothing here that appears either to support or disprove the contention that the chromosome has essentially an alveolar structure. If the sodium phosphate removes the chromatin from a homogeneous achromatic core and the chromatin is the alveolated substance, we could thus account for the vacuolate appearance. Similarly the globular masses seen in the late prophase might be considered as large alveoli, the chromatin not being completely removed. This argument, however, is not so convincing as the chromonema hypothesis.

At any rate it seems clear that there is a substratum of achromatic material that has no orderly arrangement in the resting nucleus, but assumes a regular shape in the late prophase. It is also evident that the chromatic element is soluble in sodium phosphate, leaving no evidence of itself in the treated chromosome that is visible as such, and no anastomosing strands in the resting nucleus. These substances do not seem to have the orderly arrangement demanded of the physical basis of Mendelian heredity by the facts of genetics. The visible chromosome may even be thought of as an evanescent structure, being produced and then disappearing in each mitosis, as claimed by DELLA VALLE (**8**).

If we admit this assumption, however, we must account for the facts of the persistence of the chromosomes in developing the same sizes, shapes, and numbers in each mitosis, and also the persistence of loss or gain in numbers that occurs in chromosome aberrations. Might not these things justly be attributed to the influence of the ultramicroscopic thread of genes, of whose existence the geneticists have produced such a wealth of evidence?

These threads may be considered as placed axially, in which case neither alveoli nor chromonemata are of any great practical significance; but if they are conceived as the basis of the chromonema, we can imagine how they might function under these conditions. Depending on the fluctuating attraction of these threads for chromatin, the chromonemata would change in visibility as is claimed. If the attraction of certain genes or groups of genes for chromatin were greater than that of others, lumps of chromatin (chromomeres)

might appear on the chromonema. It is conceivable that in some forms these might be arranged in a regular way, and of course they would always appear the same in the same chromosome. WENRICHS' (32) well known work on *Phrynotettix* is a case in point.

This thread of genes would be the basis for the periodic building up and dissolution of the structures we know as chromosomes. Longitudinal splitting would be a function of the gene thread, occurring at a time when its attraction for chromatin is slight, thus making its visibility very low. The other materials which flow out in anastomosing strands at times, and at least appear to accumulate in the nucleolus and later flow back, must be homogeneous and therefore not divided in any precise or qualitative way at mitosis. The view that at least the chromatic material is not the physical basis of Mendelian heredity is gaining ground, and is expressed in two recent publications (LATTER 16, WILSON 33).

The twisting about each other of homologous chromosomes during meiosis and of split halves in mitosis, and the spiral twisting reported for the chromonemata, might all be related phenomena. If the genes are regarded as the basis of the chromonema, and considered as restricted to division at each mitosis in a plane parallel to the long axis of the chromosome, they may be thought of as dividing in every vertical plane in a haphazard way. Mutual repulsion of like products of division would tend to separate them as far as possible. But since the genes are regarded as arranged in linear order and presumably attached to each other in some way, since they maintain this arrangement, separation will not be a simple matter, the longitudinal split having occurred in various planes. On this account the two new threads would be thrown into the semblance of a spiral, although twists in one direction at certain places would largely be compensated by twists elsewhere the opposite way. This twisting with repulsion would greatly shorten the distance the threads cover, thus producing the chromosome as we know it. Moreover, since the spiral is then more apparent than real, disentangling of the threads at the next prophase would be relatively simple.

This proposal is based on KAUFMANN'S claim that the daughter chromonemata are not found in separate chromosomes until a whole nuclear cycle after the split occurs. It may indeed explain why that

should be. If the split occurs in the prophase when the visible chromosome is formed with its tough peripheral layer, the split halves could not escape until after the next telophase, when the visible chromosome is dissolved; therefore we should find a dual thread in metaphase, anaphase, and telophase.

The twisting of homologous chromosomes, and of the newly formed products of division about each other, may perhaps be considered as a manifestation of gene attractions and repulsions at certain points on opposite chromosomes, thus simulating gene arrangement in a somewhat spiral way within the chromosome.

Finally, there is the problem of the functions of the visible parts of the chromosomes, if the ultramicroscopic thread is considered the essential part. Obviously they must form the internal environment of the genes. It is this material, or part of it, that the chemist has examined and found so stable and uniform, with but minor changes in protein constituent from one kind of plant or animal to another. The question arises as to what would happen to a form if a change were to occur in one of these protein constituents. Naturally the new internal environment would affect the reactions of the genes profoundly, and the more specialized the form the less likely would it be to survive the shock. The more simple forms would be better able to survive, but probably would show major changes in structure and function.

If we consider the course of evolution, we see that the great changes probably occurred long ago from relatively simple prototypes, followed by many minor changes of a specializing nature. The desmids and diatoms, the red algae, the mosses, the modern ferns, and many other such groups appear to be closed lines. Evolution is thus conceived tentatively as proceeding along two lines: great changes which are successful only in unspecialized forms, and minor changes involving specialization and rigidity. That the latter are gene mutations is indicated by the evidence of all such mutations whose origin is known. Not only do they involve special structures and functions, but nearly always they reduce the vigor of the plant or animal involved. As MULLER (**24**) states:

> Most mutations are deleterious in their effects. This applies not only to the organism as a whole, but also to the development of any particular part: the deli-

cate mechanisms for producing characters are more likely to be upset than strengthened, so that mutations should more often result in apparent losses or retrogressions than in "progressive" changes. This is both a priori expectation and a phenomenon generally observed.

The more disadvantageous mutations are eliminated through the failure of their possessors in the struggle of existence, although many such, if they are recessive, may persist in cross-bred forms. Others may survive if they can find or are subjected to peculiar environmental conditions to which they may thus seem to be adapted. The net result of gene mutations, therefore, is not so much deterioration as specialization.

As to the origin of the genes we have no information, but in the light of the nature of gene mutation the following quotation from MORGAN (23) seems significant:

> If the same number of genes is present in a white blood corpuscle as in all the other cells of the body that constitute a mammal, and if the former makes only an amoeba-like cell and the rest collectively a man, it scarcely seems necessary to postulate fewer genes for an amoeba or more for a man.

Summary

1. Sections of *Vicia faba* root tips were treated with solutions of tribasic sodium phosphate, acid sodium phosphate, sodium phosphate at pH 5, sodium hydroxide, and pepsin hydrochloric acid respectively.

2. Tribasic sodium phosphate and sodium hydroxide each caused a swelling of the nucleus and chromosomes, and almost eliminated their staining capacity. The effect of the former reagent is the greater. It is considered that the chromatin is dissolved and removed.

3. The nucleolus is shown to consist of two elements, a peripheral and central, the former probably contributing to the formation of the chromosomes.

4. A theory is proposed to account for chromonemata as indicating the presence of a thread of ultramicroscopic genes whose split halves mutually repel each other within the chromosome.

5. The suggestion is made that the visible parts of the chromosome and nucleus form an internal environment for the interaction of the genes, but are not themselves the physical basis of Mendelian heredity.

6. Changes in the constitution of this visible matter might produce a profound effect that only unspecialized forms could survive, gene mutations being responsible for minor changes involving specialization.

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VARIATION IN SUGAR CONTENT IN POTATO TUBERS CAUSED BY WOUNDING AND ITS POSSIBLE RELATION TO RESPIRATION[1]

E. F. HOPKINS

(WITH FOUR FIGURES)

While investigating the effect of low temperatures on respiration and carbohydrate changes in potato tubers, it was found by chance that wounding of potato tubers causes a marked change in the sugar content in a short period of time. The particular experiment which showed this was being carried out to gain some idea of the amount of variation to be expected in the chemical analyses due to sampling.

In preparing four samples of ten tubers each for chemical analysis, two cylinders were cut from each tuber with a cork borer, to be used for catalase determinations. The remainder of the same in each case was ground to a pulp and sampled for sugar analysis and moisture determinations. Due to lack of time it was not possible to grind these samples promptly, and therefore several of them were held for a time with cylinders cut from them (that is, in a wounded condition) before being sampled. Sample I was ground for analysis early in the afternoon shortly after wounding; sample II was ground late this same afternoon; sample III the next morning; and sample IV the afternoon of the second day. The results of the sugar analyses and moisture determinations are shown in table I.

Experiment I

The results of the sugar determinations on the wet basis are seen to increase progressively from sample I to sample IV. That this increase is not due to their increase in total solids caused by drying is evident when the data are calculated to the dry basis. In approximately one day the sugar content has increased from 2.302 to 3.400 per cent, according to the dry basis. The experiment, therefore,

[1] This investigation was carried out at Cornell University under a National Research Fellowship in the Biological Sciences.

while giving no data on error in sampling, because of delay in preparing samples after wounding, does bring out the interesting information that there is marked increase in sugar content in potato tubers following wounding.

It is well known that wounding causes an increase in the rate of respiration. This effect was first observed by BÖHM (**2**), and was later confirmed by STICH (**17**), who brought out that if the cut surfaces of the tubers were sealed together with neutral gelatin, immediately after wounding, the increase in respiration was not so great. RICHARDS (**15**) studied the effect of wounding on respiration more extensively, and has shown that an increase in respiration follows

TABLE I

INCREASE IN SUGAR CONTENT AFTER WOUNDING

SAMPLE	PERCENTAGE SOLIDS	PERCENTAGE SUGAR, WET BASIS		PERCENTAGE SUGAR, DRY BASIS	
		Reducing	Total	Reducing	Total
I	20.79	0.169	0.478	0.812	2.302
II	21.87	0.147	0.520	0.673	2.379
III	24.59	0.185	0.648	0.752	2.636
IV	23.60	0.261	0.802	1.105	3.400

wounding in potato tubers as well as in fleshy organs of other plants. In general he obtained a respiration-time curve which rises rapidly to a maximum the second hour after wounding, declines, and rises more gradually to a second maximum at the end of about 24–30 hours. There is a gradual decline from this second maximum.

It occurred to the writer, after obtaining these results, that it would be of interest to determine whether the increased respiration obtained by RICHARDS could be correlated with these changes in sugar content. Various investigators have found that the addition of sugar to culture media which come in contact with the material concerned or in which plants are grown influences respiration markedly. KOSINSKI (**6**) showed for *Aspergillus niger* a striking effect of sugar on respiration. When he washed out the nutrient solution in which this fungus was grown with the same nutrient solution minus sugar, but made isotonic by the addition of NaCl, respiration fell to a low

value. On the addition of the normal nutrient it rose again even higher than the original value. PALLADIN and KOMLEFF (12) found that etiolated bean leaves left with the petioles in sugar solutions respired much more rapidly when transferred to pure water. PURIEVITCH (14) demonstrated in the case of *A. niger* that as the concentration of sucrose increases from 1 to 25 per cent, the respiratory ratio $\frac{CO_2}{O_2}$ passes through a maximum when the sugar concentration is about 10 per cent. Perhaps the most complete paper on the effect of sugar on respiration is that of MAIGE and NICOLAS (9), who have determined the effect of varying the concentration of a considerable number of sugars on respiration. They have shown that, in general, as the concentration of the sugar in the medium increases respiration increases, until very high concentrations are reached, when partial plasmolysis occurs and respiration drops. KNUDSON (5), in his work on the effect of carbohydrates on green plants in pure culture, showed that the addition of maltose, glucose, or sucrose increased the amount of carbon dioxide markedly over that produced by the checks to which no sugar was added. He states that while this is partly due to the greater root development, it is also due to a higher rate of respiration. In these papers the internal concentration of sugar was not determined, although MAIGE and NICOLAS rightly point out that the cellular concentration of sugar is the important factor concerned with respiration, not the amount added to the culture medium.

In the case of the potato tuber we have an organism in which the cellular concentration of sugar may change either by hydrolysis of starch to sugar or by synthesis of starch from sugar, these changes being brought about by various causes. Hydrolysis of starch to sugar is brought about by keeping the tubers at 0° C., and MÜLLER-THURGAU (10) explains the increased respiration of tubers previously held at 0° C. over those held at a higher temperature by this increased sugar content. The writer also believes that this explains the results of his own experiments (3), in which the respiration at 0° was found to be greater than at 4° C.

As it appeared from the preceding experiments that wounding potato tubers also increases their sugar content, other experiments

were performed to confirm the data, and to correlate if possible the changes in sugar concentration with the changes in respiration obtained by RICHARDS.

Experiment II

A number of rather small tubers were selected and kept at laboratory temperature, which varied from 20°–22° C., for several days. The respiration of six of these tubers was determined. The next day the respiration of six other tubers was determined, and these tubers were then reduced to a pulp and the latter sampled for sugar analysis and for moisture determinations. At this time the rest of the potatoes were wounded by cutting two cylindrical plugs from each. At certain intervals samples of six of the wounded tubers were placed in the respiration chamber and their respiration determined. They were then sampled for analysis as in the case of the unwounded tubers.

The respiration apparatus used was similar in principle to that used by the writer in previous work on the effect of low temperatures on respiration (3), but on a smaller scale. The respiration chamber consists of a one-quart fruit jar fitted with a no. 12 stopper, through which pass inlet and outlet tubes. The absorption unit is made up of a 500 cc. Erlenmeyer flask, fitted with a rubber stopper through which pass an inlet and an absorption tube. This is a 50 cc. burette tube containing beads in the lower portion, and fitted with a small rubber stopper and outlet tube. The apparatus is guarded from atmospheric carbon dioxide by means of a soda-lime tower. Barium hydrate solution tubes are inserted before the respiration chamber and after the absorption unit, as precautionary tests. In none of the runs was there noted the entrance of atmospheric carbon dioxide or the loss of carbon dioxide respired, through the absorption tower. The current of air was produced by a Richards pump. During this experiment the respiration chamber was immersed in a water bath kept at 20° C. Before each run the precaution was taken to aerate the apparatus with carbon dioxide-free air.

The carbon dioxide was absorbed in 25 cc. of N/10 NaOH solution, to which had been added 5 cc. of a solution which contained 1 gm. of $BaCl_2$. At the conclusion of a run the solution in the tower was allowed to drain into the flask, and the tower rinsed with carbon

dioxide-free water which was allowed to drain into the flask. The excess of alkali was then titrated in the same flask with N/10 HCl in the presence of ortho-cresol phthalein. As in previous work, a blank was run on the alkali plus the $BaCl_2$. The difference between the blank and the titration after absorption represents the carbon dioxide absorbed in terms of N/10 acid. The end points in the titrations were accurate to one drop of N/10 acid.

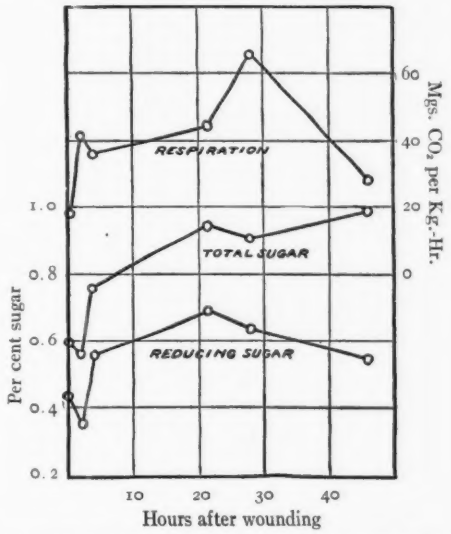

FIG. 1.—Changes in respiration, total sugar, and reducing sugar after wounding; experiment II.

The sugar analyses and moisture determinations were carried out as previously described (3).

The results obtained in experiment II are summarized in table II. It will be noted that there is good agreement in the respiration data on two different lots of tubers before wounding. The gradual increase in total solids is due to evaporation from the cut surfaces in the somewhat dry atmosphere. The data are shown graphically in fig. 1. The respiration curve is in general the same as those obtained by RICHARDS for potatoes. The marked increase in both reducing

sugars and total sugar found in experiment I was again noted. The reducing sugar appears to reach a maximum at the end of about 2

TABLE II

EFFECT OF WOUNDING ON RESPIRATION AND SUGAR CONCENTRATION IN POTATO TUBERS

DATE	HOUR	HOURS AFTER WOUNDING	WEIGHT OF TUBERS (GM.)	RESPIRATION MG. CO_2 PER KG PER HOUR	SUGAR CONCENTRATION PERCENTAGE, MOIST BASIS		PERCENTAGE SOLIDS
					Reducing	Total	
11–5–24	4:56 P.M.	0	457	18.4	.....	.....	.....
6–24	11:35 A.M.	0	420	18.7	0.440	0.600	23.87
	2:10 P.M.	2	336	41.7	0.358	0.566	23.22
	4:12 P.M.	4	347.5	36.1	0.562	0.768	23.71
	7:24 A.M.	21.5	300	44.7	0.693	0.957	24.33
	1:54 P.M.	26	255	54.4	.....	.....	.....
	3:54 P.M.	28	300	66.1	0.642	0.912	25.20
8–24	10:15 A.M.	46	322	29.0	0.558	0.995	25.94

hours, and then falls off, but the total sugar at the end of the experiment was still increasing. In general the results show that the increase in wounding is accompanied by an increase in sugar content, and that these two phenomena are approximately correlated.

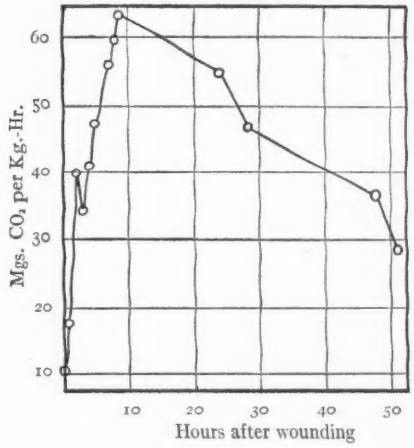

FIG. 2.—Changes in respiration after wounding; experiment III.

Experiment III

In this experiment only the respiration was studied. Two runs were made on the normal tubers, and then a series of determinations of CO_2 carried out on the same material after wounding. The data are shown in table III. Again these results are similar to those of RICHARDS, with the second maximum, however, falling probably between eight and twenty-four hours after wounding. The first maximum ap-

pears as before at the end of the second hour. The results for the first fifty-one hours are shown graphically in fig. 2.

TABLE III

EFFECT OF WOUNDING ON RESPIRATION OF POTATO TUBERS

DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION MG. PER KG PER HOUR
11–13–24	11:00 A.M.	0	9.54
	12:08 P.M.	0	10.47
	1:21	1	17.74
	2:21	2	39.95
	3:31	3	34.61
	4:21	4	41.18
	5:21	5	47.40
	7:21	7	56.36
	8:21	8	59.68
	8:51	8½	63.86
11–14–24	12:01	24	55.02
	4:51	28½	46.82
11–15–24	12:30	48	36.75
	3:15	51	28.61
11–18–24	12:21	120	25.94

TABLE IV

EFFECT OF WOUNDING ON RESPIRATION

DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION CO_2 MG. PER KG HOUR	DATE	HOUR	HOURS AFTER WOUNDING	RESPIRATION CO_2 MG. PER KG HOUR
12–1–24	12:19 P.M.	0	16.0	12–2–24	8:00 P.M.	13	35.1
	2:19	0	16.5		9:00	14	38.9
	4:19	0	16.2	12–3–24	10:05 A.M.	26	40.8
12–2–24	8:25 A.M.	½	40.7		11:05	27	42.5
	8:55	1	42.6		12:05 P.M.	28	40.1
	9:25	1½	47.4		2:30	30½	42.4
	9:55	2	34.3	12–4–24	4:30	32½	39.4
	10:25	2½	44.1	12–5–24	4:30	56	38.7
	10:55	3	46.4		12:05	76	33.0
	11:25	3½	45.3		2:05	78	34.4
	12:25	4½	46.8	12–6–24	4:05	80	33.6
	2:25 P.M.	6½	46.7		12:00 M.	100	28.3
	7:00	12	43.9				

Experiment IV

This experiment was carried out as experiment II, with the following exceptions. The respiration apparatus was placed in the chamber of an incubator where the temperature was maintained constant at 25° C. The wounded tubers for analysis were placed in a

large desiccator in the same incubator, and aerated by means of a current of moist air to prevent their drying out. The same wounded sample was used throughout the experiment for respiration deter-

TABLE V

EFFECT OF WOUNDING ON SUGAR CONTENT OF POTATO TUBER

DATE	HOUR	HOURS AFTER WOUNDING	PERCENTAGE SUGAR, MOIST BASIS	
			Reducing	Total
12–1–24	.....	0	0.261	0.458
12–2–24	8:45 A.M.	1	0.314	0.522
	11:20	3½	0.374	0.563
	2:17	6½	0.235	0.406
	4:07	8⅓	0.356	0.566
	7:40	12	0.298	0.502
12–3–24	9:45 A.M.	26	0.258	0.513
12–4–24	10:20	50½	0.334	0.638
12–6–24	10:00	99	0.427	0.699
12–8–24	1:35 P.M.	151	0.306	0.581

minations, and samples were taken from the desiccator for analysis at more frequent intervals. By continuing the experiment over a longer time, the approximate time of maximum sugar content was also determined. The respiration data are presented in table IV, and the result of sugar analysis in table V.

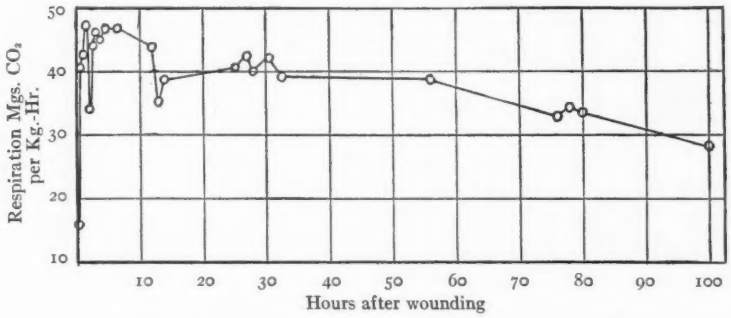

FIG. 3.—Changes in respiration after wounding; experiment IV

From table IV it will be observed that the respiration, very constant before wounding, rises rapidly as soon as wounding takes place, reaching a maximum at the end of one and one half hours; it drops off

at the end of the second hour but rises rapidly again and then gradually falls off. Fig. 3 shows the general shape of the curve. The second maximum in this case occurs even somewhat earlier than in the foregoing experiment. The curve, however, is somewhat flattened.

The data for the sugar analyses are plotted in fig. 4. It is seen that the curves also show a double maxima, the first three to six hours after wounding, and the second four days. The curves for reducing and total sugar parallel each other throughout.

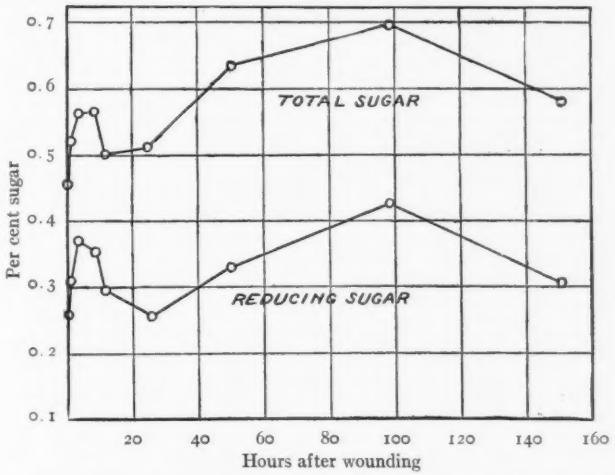

FIG. 4.—Changes in total reducing sugars after wounding; experiment IV

Discussion

From the experimental data presented there is no doubt that the sugar content of the potato increases on wounding. Considering only the total sugar, the maximum increase in experiment I is 68 per cent, in experiment II 66 per cent, in experiment IV 53 per cent. The exact course of this sugar concentration change the writer believes would have to be ascertained by a greater number of experiments; in fact, it probably will vary with the variety and condition of the tubers and possibly with the relative humidity under which they are kept after wounding. According to APPEL (1), suberization is more rapid if the wounded tubers are kept in a humid atmosphere, and, as

the writer hopes to bring out in this discussion, sugar formation after wounding is related to suberization and cork formation. The data for experiment IV, however, show a definite trend and are suggestive.

The increase in sugar concentration is associated no doubt with the general activities brought about in the tubers by wounding, which lead to the formation of suberin on the cut surfaces and to the formation of a new meristem from the parenchyma in this region. PRIESTLY and WOFFENDEN (13) have discussed the factors concerned in the formation of this cork layer. During the course of one experiment the writer sectioned some of the material which had been wounded about five days previously, and observed a clear area in the tissue extending about 2 mm. in from the wounded surface. Microscopical study showed the cells in this region, usually packed with starch, were practically free from it. The meristem as described by PRIESTLY and WOFFENDEN was evident in about the center of this clear area, no starch being observed between the suberin and the meristem. On the extreme outer surface beyond the suberin layer some starch grains were noted, which had possibly been cut off from the diastatic action by the layer of suberin. It was interesting to observe the details of cell structure in cells free from the starch which usually obscures them. In many cells the nucleus and nucleolus stood out clearly, and delicate radiating strands of cytoplasm were seen; in other cells a beautiful foamlike structure of the cytoplasm was present. In a recent paper NAKANO (11) also mentions the absence of starch in this area adjacent to the wounded surface.

One would expect, therefore, to find after wounding a higher concentration of sugar near the wounded surface, than in the tuber as a whole. It will be recalled that the analyses reported are on the basis of the tubers from which two cylindrical plugs had been removed. In order to test this idea, some of the cylindrical plugs cut from the tubers in experiment IV were kept and analyzed after several days. These plugs, having a greater proportion of wounded surface per unit volume of tissue, should have a greater sugar content than wounded tubers sampled for analysis at the same time. They had in fact a higher content of both reducing and total sugar than any of the wounded tuber samples. The results are given in table VI.

In this connection an experiment of SCHNEIDER-ORELLI (16) is pertinent. He made a qualitative test with Fehling's solution on

tubers which had been wounded four days previously, and obtained a much stronger test in this clear region near the wounded surface.

As brought out previously, there is a correlation between cellular sugar concentration and respiration, and in the case of wounding one would expect that the increased sugar content would cause an increase in respiration of wounded potato tubers. One objection to this view, of course, would be the fact that RICHARDS in his experiment obtained increases in respiration on wounding beets and other storage organs, which have no starch reserve as in the potato. If one examines the data in these instances it will be noted that the increases are

TABLE VI

SHOWING GREATER INCREASE IN SUGAR CONTENT NEAR WOUNDED SURFACE

	PERCENTAGE SUGAR	PERCENTAGE INCREASE OVER UNWOUNDED TUBERS
Cylinders		
Reducing sugar	0.486	86
Total sugar	0.735	60
Wounded tubers		
Reducing sugar	0.427	64
Total sugar	0.699	52

not so large, and the second respiration maximum is not so marked. It is entirely possible that here the increased respiration is due entirely to the changes brought about by the diminution in the carbon dioxide concentration in the tissues. MAGNESS and DIEHL (8) have shown that coating apples with paraffin or oil causes a reduced respiration rate. At the same time they show, by analysis of the intercellular air, that while there is sufficient oxygen, carbon dioxide accumulates. They ascribe the reduced respiration rate to this accumulation of CO_2. Conversely one might assume that a diminution of the CO_2 concentration would accelerate respiration. RICHARDS himself states that the results were not so striking as in the experiments with potatoes and carrots. On the other hand, one cannot entirely account for the increase in respiration in potatoes on this basis, when those experiments of RICHARDS are considered where the wounded parts were immediately stuck together by means of clay after being cut. In these cases the initial maxima were absent, but the secondary

maxima, although not so great, were very marked. Here the sugar produced in the process of wound healing is probably a factor.

In one of RICHARDS' experiments, for example, the respiration of 225 gm. of potato tubers was determined and found to be less than 1 mg. per hour. They were then halved and immediately stuck together again with neutral clay. No preliminary maximum occurred, which is in accord with RICHARDS' idea that there is no rapid outward diffusion of CO_2; but the respiration gradually increased, until at the end of 24 hours the rate was 7.5 mg. per hour. At the end of 55 hours it was 2.5 mg. per hour. While this is by no means a large increase, yet compared with other experiments in which the cut parts were not stuck together with clay it is a large increase, probably at the greatest being more than eight times the rate before wounding.

It should be pointed out that, when the cut parts are stuck together with neutral clay (as in the experiments of RICHARDS), although the air is excluded and suberization does not occur, meristem or cork cambium formation takes place (**13**), with the accompanying hydrolysis of starch to sugars.

MAGNESS (**7**) has suggested that part of the increased respiration following wounding is due to a mechanical facilitation of the gaseous exchange and part to metabolic changes in the wounded tissue. JOHNSTONE (**4**) concludes, from an experiment of STICH (**17**) on potato tubers, that 120.9 per cent of the increase obtained in the rate after wounding is due to injury, and 376.7 per cent is due to the facilitation of gaseous exchange. In his own experiments with sweet potatoes, he found 97.3 per cent increase in respiration when cut parts were exposed, and 17.15 per cent when they were immediately sealed together after cutting.

When measuring respiration by means of gas exchange, it should be pointed out that other factors should be considered in interpreting results. For instance, as soon as wounding occurs, processes are set in action which produce suberization of the wounded surfaces, and thus tend immediately to cut down gaseous exchange. Even if we postulate a direct effect of sugar concentration on respiration, therefore, we must expect the maximum in sugar concentration to lag behind the point of maximum respiration. At the same time that the sugar content is causing an increase in the respiration, suberization of the wound is cutting down gaseous exchange, causing an increase

in the amount of carbon dioxide in the tissues, which has been shown to lower the respiratory rate (**8**). On this basis the explanation of the results obtained in experiments II and IV is obvious. In both of these experiments the total sugar curve is still rising after the maximum respiration point has been passed.

Perhaps too close a correlation should not be drawn from the limited data presented, but in general it is true that with increased sugar the respiration is augmented. Even after considerable time has elapsed following wounding and callus formation, respiration is still much greater than before wounding, both in my experiments and in those of RICHARDS. At the time the sugar content is still higher than in the unwounded state.

In experiment IV the preliminary maximum at the end of a few hours is interesting, and may be caused by a disturbance of equilibrium conditions in metabolism, brought about by the sudden release of carbon dioxide from the tissues which RICHARDS has shown to take place when the tubers are first cut.

Summary

1. A marked increase in sugar content is shown to follow wounding. The maximum increase found in the experiments reported varied from 53 to 68 per cent of the original sugar content.

2. In general the sugar content rises to a maximum after wounding, reaching a high point after several days, and again falls off. It is possible that there is a preliminary maximum not so great as the other at the end of a few hours.

3. From microscopical observations and chemical analyses this increase in sugar content is thought to be brought about by activities leading to callus formation.

4. The augmentation of respiration which follows wounding can be explained logically on the basis of the increase in the sugar content of the cells, although it is pointed out that because of other factors the curves for respiration and sugar content will not be exactly parallel.

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ABNORMAL SEX ORGANS OF MNIUM MEDIUM

GEORGE S. BRYAN

(WITH TWENTY FIGURES)

Introduction

The discovery of numerous examples of abnormal sex organs in *Mnium medium* has made it seem worth while to investigate carefully the details of these peculiar structures.

LINDBERG (**5**) has reported that in 1878, while examining samples of female plants of *Hypnum*, certain perichaetia were noted which, in respect to the length and breadth of their leaves, stood midway between male and female perichaetia. On closer inspection sex organs were found, some of which resembled archegonia in structure, while others bore a closer resemblance to antheridia, so that one might adduce a series of transformations from actual female to actual male organs. The venter of a transformed archegonium was observed filled with extremely fine grained material, perfectly resembling a dried mass of spermatozoids; but LINDBERG was unwilling to commit himself that this dried mass was actually equivalent to such. No rounded egg could be seen in the venter of any transformed organ. A plate of drawings showing the external appearance of these organs accompanies the article.

HY (**4**), in a paper on the archegonium of the mosses, mentions that different species of mosses have archegonia transformed at their summit into antheridia. He states that *Atrichum undulatum* is one of the species in which this phenomenon is most frequent, especially in the synoicous inflorescences terminating a stem two years old. No figures are given to illustrate the statements.

DE BERGEVIN (**2**) has reported specimens of *Plagiothecium sylvaticum* with marked peculiarities in the forms of the sex organs. These are borne in groups along the lower portions of the branches of a plant. For the most part these groups were composed of antheridia only, but each of a few groups consisted of an archegonium surround-

ed by two to several antheridia. In some groups DE BERGEVIN found what he termed archegonia being transformed into antheridia: "En réalité, il y a là une véritable interversion dans le process organique." A series of sketches showing in outline form these transformations from archegonia to antheridia accompanies the article. In explanation of this phenomenon of transformation, DE BERGEVIN advances the hypothesis that shade and moisture are the controlling factors. When the conditions of shade and humidity remain below a certain limit only archegonia are produced; when this limit is exceeded archegonia give place to antheridia. The intermediate condition favors synoicous plants. DE BERGEVIN is silent on the question of the actual formation of antherozoids in the transformed antheridia. He merely asks the question, "Les anthéridies anormales sont elles capables de féconder l'organe femelle?"

HOLFERTY (3), in an account of the development of the archegonium of *Mnium cuspidatum*, has briefly described a number of abnormal organs that "constitute a progressive series leading from the normal archegonium to the normal antheridium." Several drawings illustrate the structure of these organs as seen in section. HOLFERTY speaks of sperm mother cells as being present in one of the organs, but does not discuss the actual formation of antherozoids.

Shortly after the appearance of HOLFERTY'S paper, LYON (6), in an article on the evolution of sex organs in plants, makes brief mention of HOLFERTY'S paper, and adds that "specimens have been secured with perfectly typical moss sperms which were discharged from the antheridial region above the egg."

Material and methods

The material for this study was gathered for several years in succession from a bank having a northern exposure on the shore of Lake Mendota, near Eagle Heights, and was supplemented by collections from the Dells of the Wisconsin River. The plants from both localities have been identified by Mrs. ELIZABETH G. BRITTON as *Mnium medium* (Bryol. Eur.). It is interesting to note that the material from these two stations, approximately 60 miles apart (the only localities in which thus far I have been able to find *M. medium*), showed about equal numbers of abnormal organs. Since these ab-

normal organs have regularly recurred for a number of years in both of these stations, it seems probable that they are a constant feature of *M. medium*.

Various killing agents were used, but the most satisfactory results were obtained with Flemming's medium, and with Rawlins' formal-acetic-alcohol. After imbedding in paraffin, serial microtome sections were cut 5–12 μ in thickness. As stains, safranin in combination with Licht Grün, and Flemming's triple were employed. Considerable difficulty has been experienced in sectioning mature plants, since the usual process of imbedding renders them hard and brittle.

Development of normal sex organs

Mnium medium is synoicous. Many hundreds of heads have been studied, and thus far no exception to this statement has been found.

In the spring of the year, among the old branches, two kinds of new branches make their appearance. The purely vegetative branches grow long and slender, have a small bud at the apex of the branch, and quickly tend to assume a dorsiventral position. The gametophores, on the contrary, are short and stout, have a relatively large bud at the apex of the branch, and always grow directly upright.

As is usually the case among synoicous mosses, the antheridia appear first. The first antheridium arises from an immediate segment of the apical cell. Very quickly the apical cell itself becomes involved in the production of other antheridia, so that by the time four or five antheridia have been formed the characteristic structure of the apical cell has been lost, and a broadening region of relatively large irregular cells takes its place. From this meristematic area other antheridia arise.

The antheridium in its development appears to follow the general method already known as characteristic of the Bryales. After a variable number of antheridia (frequently a dozen or more) have been formed, archegonia begin to develop from the cells of the central portion of the meristematic region, which has now grown into a relatively broad, flat-topped receptacle. As a result of this development process, there is a general tendency toward a definite arrangement of the sex organs: a central group of archegonia, and a peripheral group of antheridia. A careful study, however, of the distribution of the

sex organs on a large number of receptacles has shown that there are exceptions to this general tendency, and that the organs are not infrequently intermingled. Thus occasionally an archegonium has been observed among the peripheral antheridia, and young antheridia have often been found growing among the centrally placed cluster of maturing archegonia.

The development of the archegonium has been carefully followed, and in practically all points it conforms with my study (1) of the development of the archegonium of *Catharinea angustata* Brid. A peculiar cytological detail, which was not found in similar organs of *Catharinea*, appears in the archegonia of *Mnium medium*. In the cytoplasm of young egg cells there are always present one or more small bodies, which in general appearance and in staining reaction resemble nucleoli. As the egg cells grow older these bodies increase in size, and, in case there are several present, undoubtedly unite, since but a single body, often exceeding in size the nucleole of the egg nucleus, is generally to be found in the cytoplasm of the egg cell at or near maturity.

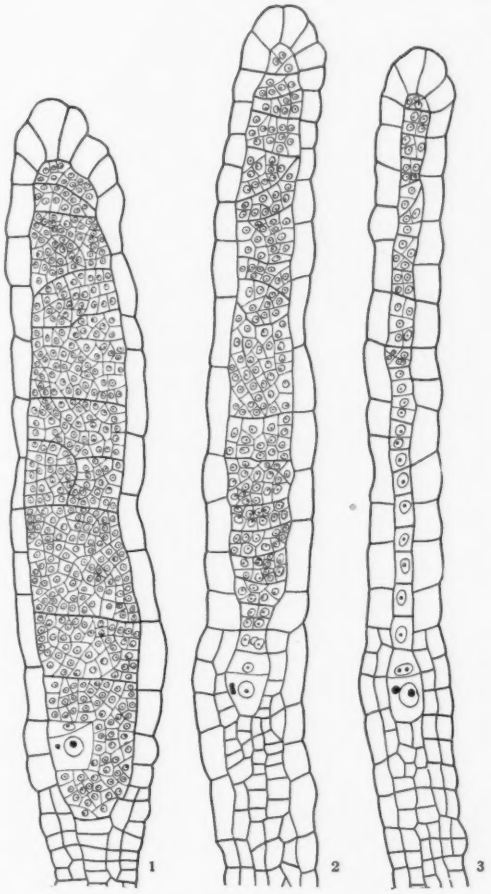
These bodies have been traced back to relatively small globules in the cytoplasm of the ventral cells of the archegonia, and are sometimes quite conspicuous just prior to the division of the ventral cell into ventral canal cell and egg. The cytoplasm of the ventral canal cell rarely shows the presence of these peculiar structures, but they have always been found present in the cytoplasm of the egg. The interesting fact is that these cytoplasmic bodies are conspicuous in what appear to be egg cells in abnormal organs now to be described.

Abnormal sex organs

The great majority of maturing receptacles have shown at least one abnormal organ, and occasionally as many as four have been found on a single receptacle. Most often the abnormalities are located in the transition zone between the two rather well defined groups of sex organs; but they are by no means confined to this zone, as they have also been found among the early formed peripheral band of antheridia and among the later formed central cluster of archegonia. They may thus replace either an antheridium or an archegonium.

The abnormal sex organs have shown a wide range of variation in

the details of their structure. Some of them are very close in structure to normal antheridia, others differ but slightly from normal ar-

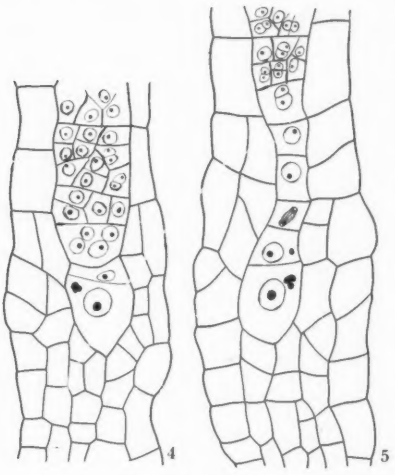


FIGS. 1–3

chegonia. Between these two extremes lie a jumble of forms, many of which are difficult to interpret as to their method of development. It is entirely unnecessary to describe and illustrate all of the varia-

tions that have been found. What appear to be some of the outstanding types have been illustrated, and are briefly described here.

Perhaps one of the most remarkable organs discovered in this investigation is that shown in fig. 1. It is apparently a typical antheridium which is approaching maturity, but at one edge in its lower part are two cells that seem to be, respectively, a ventral canal cell and an egg. Even the cytoplasmic body, which regularly accompanies typical maturing egg cells, is to be found in the cytoplasm of this probable egg cell.



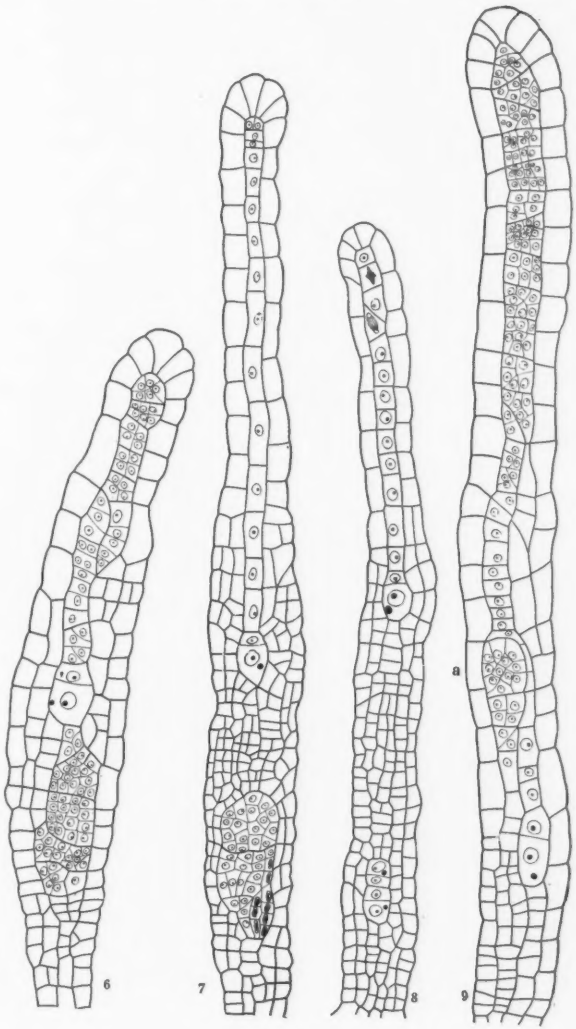
FIGS. 4, 5

Fig. 2 illustrates a type of structure which was found a number of times. The lower portion of the organ is characteristically that of the stalk and venter of an archegonium, and within the venter is a typical ventral canal cell and an egg; but above these two cells lies a structure which, except for its slenderness, is wholly antheridial in nature. In one example of this type (fig. 4) an antheridial-like group of cells is in immediate contact with the ventral canal cell, but in another case (fig. 5) several neck canal cells intervene between the ventral canal cell and the antheridial-like group of cells.

Fig. 3 is characteristic of organs that also were not infrequent in the material studied. Here without question is an archegonium typical in all respects, except that the upper part of the canal row is multiple, a condition which might be described as illustrating an antheridial tendency.

The abnormalities which have been described thus far constitute a very close and remarkable series of organs which bridge the gap between normal archegonia and normal antheridia.

Fig. 6 is rather typical of a peculiar organ that was found several times. There is an apparent venter which incloses a ventral canal



FIGS. 6–9

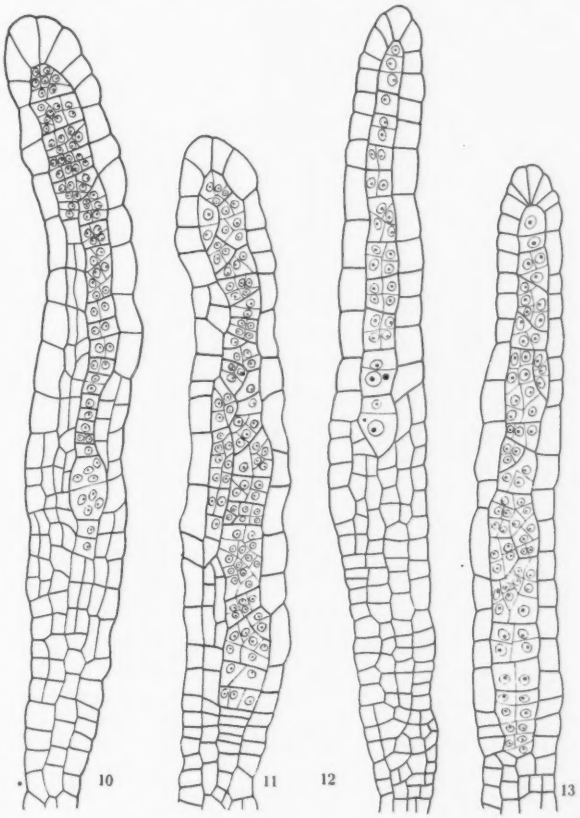
cell and an egg cell. The nucleus of the ventral canal cell, however, is somewhat larger than is generally the case, and a cytoplasmic body is present. It is probable that this ventral canal cell is a potential gamete. Above and below the venter are antheridial-like cells, the basal group particularly resembling the cells of an antheridium. Fig. 7 may possibly be regarded as belonging to the same general category as fig. 6. In any event it presents the anomaly of a well defined antheridial-like mass of cells imbedded in the elongated stalk of a typical archegonium. Two examples of this structure were found in the material studied. A very similar organ is illustrated (fig. 47) by HOLFERTY (3).

Fig. 8 is of interest as showing a gamete-forming tendency of the opposite sex in a structure similar to fig. 7. Imbedded in the lower part of the much elongated stalk of an archegonium is a double venter, the upper ventral canal cell and egg lying in an inverted position. A cytoplasmic body accompanies each probable egg cell. No indications could be found of any cells that might be interpreted as a canal row connecting the double venter with the outside. Two other structures practically identical with the one described and illustrated were found in the material.

Fig. 9 presents a curious mixture of characters. The upper portion is decidedly antheridial in nature, but merges below into an evident row of nine neck canal cells. The outline of what probably began as a venter may be noted at *a*, but no potential egg cell is present, its place being occupied by a number of small antheridial-like cells. Near the base of the organ is an evident venter containing probably two potential gametes. Fig. 10 repeats many of the details found in fig. 9. There is the same upper portion, antheridial in nature, which merges below into a row of neck canal cells. The venter is better defined than in fig. 9, but differs in that each cell within the venter of fig. 10 contains four small nuclei without walls between them.

In all of the organs previously described there has been present at least one well defined egg cell. In fig. 10 there is no indication of any such cell. Each of the eight nuclei within the venter might be interpreted as a potential egg nucleus, but the small size of each is a weighty objection to such a suggestion. With fig. 10 we pass to organs of a type characterized by the absence of any well defined prob-

able egg cell. Such organs were of frequent occurrence in the material studied, and were often found between the peripheral band of antheridia and the central cluster of archegonia. Figs. 11 and 13



FIGS. 10–13

illustrate some of the variations of detail that may be observed in such organs.

Development of abnormal organs

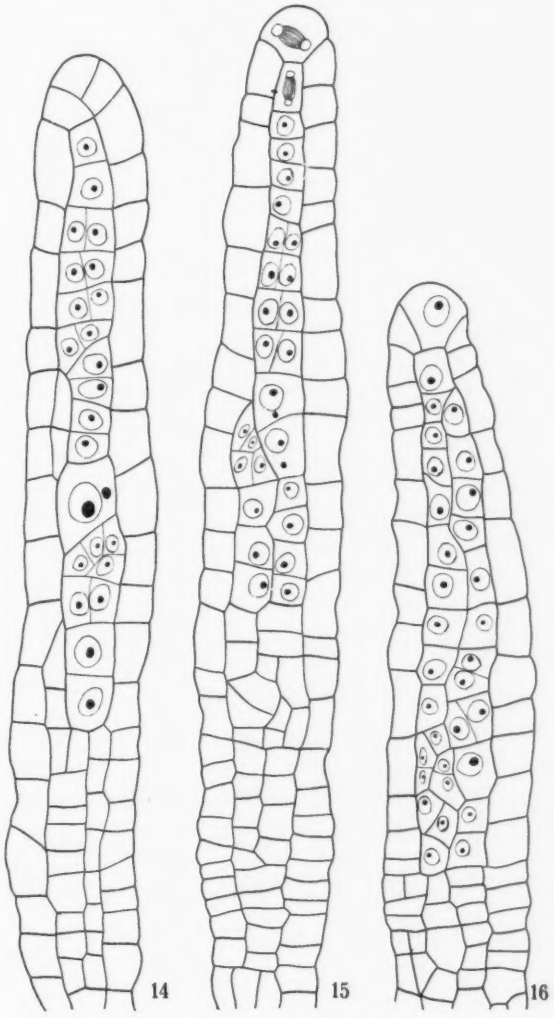
Having found certain types of abnormal organs, an effort has next been made to discover the manner in which these various types

have developed. Some of them clearly tell their own story. Thus there can be little doubt that the organ illustrated by fig. 1 has developed after the manner of a typical antheridium, and that the ventral canal cell and egg have been derived from one of the early segments. Likewise, the type illustrated by fig. 3 shows that it has developed after the method of a typical archegonium, except that the canal cells in the upper part of the neck have divided and redivided an unusual number of times. This unusual number of neck canal cells should undoubtedly be regarded as an antheridial character appearing in an archegonium. Not only do the cells in the canal row resemble in general appearance those of an antheridium, but the nuclei of a number of adjacent neck canal cells have been found to divide simultaneously, which is one of the characteristics of a developing antheridium. These facts now make clear the meaning of the multiple neck canal cells and their simultaneous division observed (1) in some of the maturing archegonia of *Catharinea angustata* Brid.

While the interpretation of the developmental processes of figs. 1 and 3 is reasonably certain and clear, it is quite otherwise with such figures as 2, 6, 9, 10, and 11. For example, in fig. 2 the general manner of development may have been that of an archegonium in which there has been a greater growth and multiplication of the neck canal cells than is shown in fig. 3. On the other hand, the apical cell of the organ may have begun its activity as of the archegonial type, and later may have changed its method of segmentation to the antheridial type, thus explaining some of the irregular divisions in the upper part of the organ. In the hope of throwing some light on this problem of development, a search has been made for early stages of these abnormal organs. Figs. 14, 15, and 16 represent some types of young organs that could be recognized with certainty. Fig. 14 is clearly a young archegonium undergoing modification. Earlier stages of this type could not be recognized with certainty, but are probably represented by certain young archegonia which have unusually broad canal cells.

Fig. 16 is a type quite different from that illustrated by fig. 14. The short stalk, and particularly the breadth and irregular arrangement of most of the potential gamete-producing cells, indicate a modified antheridium. It will be observed, however, that the apical cell

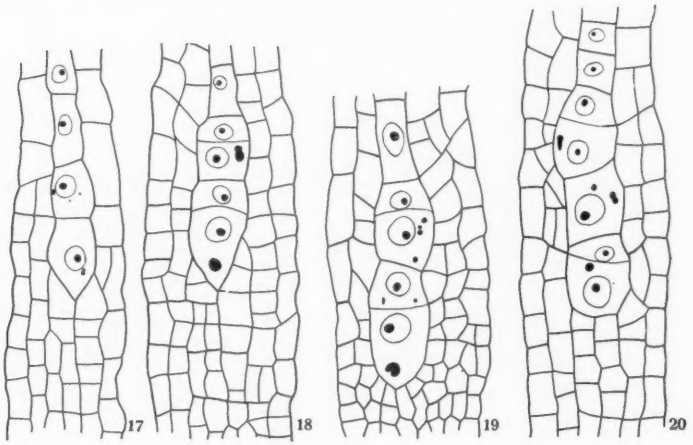
and the segment immediately beneath it are wholly archegonial in structure. It therefore seems probable that this organ began its de-



FIGS. 14-16

velopment by an apical cell of the antheridial type, and that this apical cell has only recently undergone change to the archegonial type. Fig. 13 belongs in the same category as fig. 16, in that it is undoubtedly a modified antheridium. Organs similar to fig. 13 were numerous in the material studied, and are probably represented at an early stage by what seemed to be unusually slender young antheridia.

Fig. 15 seems to belong to the same general type as fig. 16, except that the conversion of the apical cell into the archegonial type occurred at an earlier time in the developmental process.



FIGS. 17–20

The material studied contained a number of examples of double and even triple venters. The origin of a double venter is evident from an examination of fig. 17. In this young archegonium the basal neck canal cell has grown almost as large as the ventral cell. The characteristic cytoplasmic bodies are present in both cells. Fig. 18 is clearly a later stage of such a type of development. Figs. 19 and 20 seem to indicate a method by which triple venters may arise. The ventral canal cell of the lower venter of fig. 19 shows evident tendencies toward the formation of a gamete, as indicated by the size of the cell, of its nucleus, and the presence of cytoplasmic bodies. Fig. 20 probably represents a later stage of development of such a structure as is indicated by fig. 19.

Summary

1. Abnormal sex organs are apparently regularly formed by *Mnium medium*.

2. An abnormal organ may replace either an antheridium or an archegonium, but is most often found on the border between the peripheral ring of antheridia and the central group of archegonia.

3. There are wide variations in the details of structure of the abnormal organs.

4. Many of the organs have developed as modified archegonia; some as modified antheridia; and a few give indications that the apical cell has functioned for a time like that of an antheridium, and later has changed to the archegonial type.

5. A remarkable series of organs linking together antheridia and archegonia have been found.

6. The facts herein presented add further evidence to the hypothesis that the sex organs of the bryophytes are homologous structures.

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BRIEFER ARTICLES

A WATERING SYSTEM FOR CULTURE VESSELS

(WITH ONE FIGURE)

Many experiments have been carried out to determine the water requirements of plants, or the influence of the soil moisture upon their metabolism. The usual procedure has been to grow plants in soil containing varying amounts of moisture. Several watering systems have been devised and used for maintaining a uniform distribution of the moisture through the soil of the culture vessels. KIESSELBACH'S (4) coil watering device and LIVINGSTON'S (5) porous cup atmometer are perhaps the best known. Several others are described by BRIGGS and SHANTZ (1) in their summary of transpiration experiments. These systems, as well as others, seem to work satisfactorily only under certain conditions. KIBBE (3) found that, in crocks equipped with Livingston porous cup atmometers, the roots of the plants collected on the surface of the porous cups, forming a thick mat. The result was that the plants made a comparatively good growth in the "dry" soil when compared with those in medium moist soil. YUNCKER (8), using another watering system, obtained similar results. These results are quite contrary to observations in the field, where drought checks the growth to a considerable extent. SMITH (7) estimates that most crop plants suffer more from deficient water than from all other unfavorable factors combined.

SHANTZ (6) in a recent paper declares that most transpiration experiments will have to be repeated because the experimenters have not taken into account the behavior of the water in the soil. He also points out the difficulties of watering dry soil and obtaining an even distribution below the "field carrying capacity" of the soil. The problem of watering culture vessels satisfactorily seems still to be an unsolved one.

In a culture vessel the soil is relatively loose, so that water will sink into it more readily than into soil in the field. It is for this reason not quite certain whether SHANTZ'S term "field carrying capacity" of the soil is strictly applicable to soil in culture vessels. The looseness of the soil in a vessel makes watering there easier than in a field. Watering on the surface of the soil gives, within reasonable limits, a rather uniform distribution of moisture throughout the vessel for a short time, provided the water

is applied slowly. However, water applied in this manner will soon cause a hard crust to form from the top layer of the soil, provided it is not extremely light. This crust, being more compact, absorbs more water than the lower layers of the soil, and interferes with the gas exchange of the lower layers, preventing satisfactory results. Some improvement is produced by sinking a small flower pot in the center of the filled vessel and adding the water into this pot, keeping the surface of the soil unwatered; but in vessels where a uniform distribution of a smaller amount of moisture than the optimum for plant growth is required, this system does not give the desired uniformity.

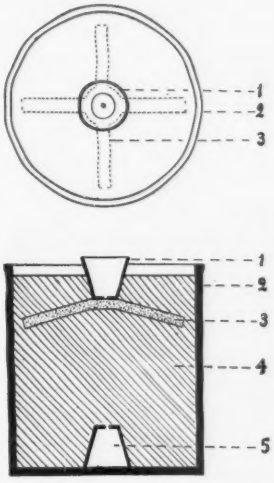

FIG. 1.—Diagram of watering system for uniform distribution in culture vessels: 1, small flower pot for watering; 2, culture crock; 3, sand for spreading water horizontally; 4, soil; 5, small flower pot serving as air space.

In an experiment in which plants were to be grown under three conditions of soil moisture, dry, medium moist, and moist, the writer found the following watering system to give a very uniform distribution of the soil moisture in the dry vessels, the ones which always have caused the most trouble. In the bottom of each vessel, which in this experiment was a one gallon glazed crock, a small flower pot was placed to serve as an air space. The necessity of this pot may be questioned. The crock was then almost filled with air-dry soil, consisting of silt loam with 10 per cent fine sand. The surface of the soil was kept higher in the center of the crock. With a finger, four radiating grooves were made in the surface of the soil and filled with coarse sand, a small flower pot was placed in the center of the cross, and the remainder of the soil added (fig. 1). The radiating arms of sand were covered with about one inch of soil. In vessels larger than those used in this experiment a larger number of radiating arms of sand may be necessary to obtain the desired result. This watering system seemed to work very satisfactorily. Three degrees of soil saturation were maintained, 15, 30, and 45 per cent of the moisture-holding capacity of the soil as determined by HILGARD'S (2) method. The plants grown were wild mustard and oats together, for the purpose of obtaining plants with an

anatomical structure modified by the degree of soil moisture. It was found that plants grown in vessels containing dry soil, compared with those grown in the medium moist soil, greatly resembled plants grown under dry and moist conditions in the field. Five dry cultures gave a yield of leaves of wild mustard of 2.9 gm. dry matter, while five cultures from the medium moist series gave 7.8 gm. yield; the leaf areas were respectively 8.83 and 32.73 sq. dm. These results seem to confirm SMITH'S statement about the necessity of water for crop growth, and do not agree with the reports of some workers who were able to obtain almost as good growth in "dry" cultures as when water was supplied liberally.

If a watering system in dry soil gives an uneven distribution of the moisture, the roots seem to develop most in the moist region of the soil in the vessel, as was the result in KIBBE'S experiments. The development of the roots seems, for that reason, to be a fair indication of the efficiency of the watering system used. Plants growing in dry soil watered by the method here described in no case showed any conspicuous development of the roots in the upper part of the soil; usually the roots were uniformly distributed throughout the whole mass. Thus this system seems to make it possible to provide a uniformly dry soil for growing plants in culture vessels.

An additional feature of this system is that it makes it possible to water the cultures quickly and easily. In vessels equipped with this watering system the water was absorbed in one minute or less, while in vessels without the arms of sand the absorption of the water required several minutes.—ALFRED ASLANDER, *Department of Botany, Cornell University.*

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NOMENCLATORIAL NOTE ON SMITHIANTHA FULGIDA (ORTG.) CHENEY, COMB. NOV.

A recent study of the Mexican and sometimes cultivated plant of greenhouses known as *Naegelia fulgida* Ortg. shows that it is incorrectly designated by the present name. The genera *Naegelia* and *Smithiantha* of the Gesneriaceae (Metachlamydeae) have been confused, due to the improper generic use of the term *Naegelia*. Investigation reveals that *Naegelia* was first employed by RABENHORST in his *Kryptogam–Flora* (85, 1844), for a genus of Saprolegniaceae. This original application of the generic name *Naegelia* is still credited as valid by the mycologists, and is listed in OUDEMAN'S *Enumeratio Fungorum* (1:111. 1919).

Apparently unaware of the chronology of publication or of the actual existence of the fungal genus of RABENHORST, one finds that several authors have described genera in various groups of plants under the term *Naegelia*. In ZOLL. and MOR. *Syst. Verz. Zoll.* (1845–46) *Naegelia* is applied to a genus of Rhamnaceae which is now synonymous with *Lupulus* O. Ktze. of the Rhamnaceae. LINDLEY used the same name (*Naegelia*) in 1845 for a genus of Rosaceae; see also LINDLEY'S *Veg. Kingdom* 560, 1847. LINDLEY'S genus is now equivalent to *Malacomeles* Dcne. The term *Naegelia* of REGEL in *Flora* **31**: 249. 1848, for a genus of Gesneriaceae was absorbed in 1891 by the *Smithiantha* of O. KUNTZE in his REV. GEN. PL. II. 977, 1891.

The species *fulgida* was described under *Naegelia* by ORTGIES, in REGEL, *Gartenfl.* 97, 1867. In consideration of the change in generic terminology making the combination *Naegelia fulgida* Ortg. impossible, and the fact that an examination of herbarium specimens substantiates the species description by ORTGIES, the combination should be **Smithiantha fulgida** (Ortg.) Cheney, comb. nov.—RALPH H. CHENEY, *Washington Square College, New York University, New York.*

Decapitalization of specific names

Commencing with this issue, the BOTANICAL GAZETTE will abandon the use of capitals for all specific names, thus meeting the wishes of an increasing number of contributors, and following the practice of Biological Abstracts and most biological journals.

CURRENT LITERATURE

BOOK REVIEWS

Plant ecology

Teachers of plant ecology will welcome the appearance of McDOUGALL's book[1] presenting an elementary treatise of this subject, and especially designed as a textbook for use in beginning classes. The work is the outgrowth of the author's experience, extending through several years, in teaching beginning classes in plant ecology at the University of Illinois, and the chapters of the book are based on the lectures prepared for these classes.

The introductory chapter defines the subject and points out the importance of such a study of plants. The next three chapters deal with the environmental relationships of the three fundamental vegetative systems of the plant, root, stem, and leaf. In the chapter on stems considerable space is devoted to a description of the various tissues of the stem. This account is necessarily very brief, and so condensed that a student without any previous knowledge of stem structure could scarcely be expected to form any adequate idea of these component tissues from the facts presented. It is to be assumed that a student of plant ecology would probably have had an introductory course in botany, and in that case this portion of the chapter would represent merely a review of the subject. It may have been so intended, but in the reviewer's judgment this material should have been replaced by a brief discussion of the structural modifications induced in these tissues by various environmental factors.

Chapters V to IX develop a very complete and thorough analytical presentation of the subject of symbiosis. Social and nutritive types of symbiosis are described for each of two larger classes of symbiotic phenomena, disjunctive and conjunctive symbiosis. Much confusion occurs in the writings of various authors dealing with this subject, and the clear elucidation of the facts recorded in these chapters should enable the student to steer clear of the uncertainty that so often develops when an attempt is made to understand the classification of symbiotic phenomena.

Chapters X to XIV deal with the five important physical factors of the plant's environment, light, temperature, air, soil, and water. The facts are tersely presented and the discussion is very much restricted, but the scope of the presentation is such that all the salient features necessary for an elementary course are included, and by making use of more comprehensive reference works

[1] McDOUGALL, W. B., Plant ecology. pp. vi+326. Philadelphia: Lea & Febiger. 1927.

the teacher can extend the discussion as much as may be desired. Chapter XV discusses the characteristics of plant forms that have developed in response to a certain uniformity of environment, and that are distinctive of plants growing in those habitats where such a degree of uniformity obtains. This chapter also includes a brief discussion of the life forms of RAUNKIAER. Chapters XVI to XX deal with synecology. The idea of plant succession is very ably presented, and the terminology used in the classification of plant groupings is clearly defined. The author is at his best in the development of this portion of the text, and these chapters reflect something of his genuine pleasure in this phase of ecological study. Chapter XXI presents the subject of phenology, with a brief discussion of certain seasonal aspects of vegetation, and chapter XXII gives an account of the distribution of some of the major plant communities, with the idea of merely introducing the subject of plant geography. Chapter XXIII is concerned with the subject of applied ecology. Keeping in mind the work of BRIGGS and SHANTZ, COWLES, SAMPSON, WEAVER, KIESSELBACH, and many others who have demonstrated the importance of ecology in its application to the solution of practical problems in agriculture, the teacher will be glad to note in the final chapter of the book the emphasis that MCDOUGALL has placed on this phase of ecological work. There is also an appendix of ten pages which gives a large number of general suggestions concerning laboratory and field work.

The volume is handsomely bound, and reveals a high grade of excellent workmanship in every detail. The text is printed in clear, bold type, and is surprisingly free from typographical errors. There is an abundance of good illustrations, well chosen to clarify and enliven the descriptions of the text.

As the first elementary American textbook in plant ecology, MCDOUGALL's book will doubtless receive its full share of criticism. There are some who will find fault with the organization of the material, but there is internal evidence in the book that such criticism already exists in the mind of the author himself. No subject has ever yet been presented in an ideal textbook, and certainly no such book may yet be anticipated in a scientific field so young as that of plant ecology. However, all must agree that MCDOUGALL has produced an excellent pioneer work, and it is to be hoped that his venture may encourage a deeper and more extended interest in the adequate presentation of the subject of plant ecology.—P. D. STRAUSBAUGH.

Enzymes

Biologists have long felt the need of a recent, authoritative book on enzymes. There are recent works which deal with the nature of enzymes and their action, but books which treat enzymes in a broad general way have been notably lacking. One has had to rely on chapters in books dealing with plant and animal chemistry.

In their recent book WAKSMAN and DAVISON[2] have done much to supply

[2] WAKSMAN, S. A., and DAVISON, W. C., Enzymes, properties, distribution, methods and distribution. pp. xii+364. Baltimore: Williams and Wilkins Co. 1926.

this need. The volume is divided into four sections. The first considers the properties of enzymes. The introductory chapter gives a clear statement of the historical development of our conception of enzymes, defines them, and gives their criteria and a table of classification. Chapter II of this section considers the rôle of enzymes and their secretion, and treats proenzymes, coenzymes, antienzymes, and the specificity of enzymes. Chapter III takes up the chemistry of enzymes and enzyme reactions. Our lack of knowledge of the actual chemical nature of enzymes is reflected in the small amount of space devoted to this subject. Chapter IV considers the factors that affect enzyme activity.

The second part of the book treats the distribution of plant and animal enzymes. The relatively large amount of work that has been done on animal enzymes is indicated by the fact that over half of the sixty pages of this section is devoted to human and animal enzymes, the other pages treating plant enzymes and enzymes of the microorganisms. Part III contains 125 pages, devoted primarily to a consideration of the methods of preparation and study of the different enzymes, but containing much material on the occurrence and mode of action of the enzymes. The different groups of enzymes as given in the table of classification are treated here. An interesting and useful section is the last one, in which is given a consideration of the practical applications of enzymes. One is impressed by the many uses to which enzymes are put in the industries and in various practical lines.

The volume is more a reference book than a textbook. The successful attempt has been made to give a concise summary and evaluation of the vast, often contradictory literature on enzymes, and thus lay the basis for future work on this subject. Important features of the book in this connection are the extensive bibliography, over 1350 references, and the large amount of space devoted to a consideration of the methods of preparation and study of enzymes. The book should be of great value to the teacher, the investigator, and the worker in the various applied fields where enzymes are used.—S. V. EATON.

Oeuvres de Pasteur

In assembling into one authoritative text the scattered papers of PASTEUR, VALLERY-RADOT[3] has performed a great service to biology in all its branches, to chemistry, and to the scholarly world generally. It is very gratifying to have the new development in physiology and bacteriology redacted and authenticated within the lifetime of men who knew the great teacher and are themselves not yet old. VALLERY-RADOT has applied himself to his task with an industry and thoroughness worthy of its magnitude, and the publishers have not lagged behind him in the typographical and mechanical work. The type has that beautiful face that only Continental designers seem able to achieve, and the reproductions of the original illustrations have been excellently and in some instances strikingly arranged. The four volumes thus far received in this country cover

[3] VALLERY-RADOT, P., Oeuvres de Pasteur. vols. 7. Paris: Masson et Cie.

PASTEUR's earlier chemical researches, his explosion of the spontaneous generation theory, studies on wine and vinegar, and the classical work on the diseases of silkworms. The three yet to come will cover his studies on beer, his treatises on virulent diseases and their prophylaxis, especially rabies, and a final volume of miscellanies. By the simple fact of its existence, this edition imperatively demands a place in every scientific library. The price is so low, only about $16 at current exchange rates for the four volumes thus far issued, that even small institutions can afford to purchase it.—F. THONE.

Plant geography

There has long been a need for a general volume on plant geography, less technical than that of SCHIMPER and DRUDE, and designed primarily for the amateur who desires an acquaintance with the vegetation of the different countries of the world. It is not unfitting that such a book has come from the hand of a plant morphologist, who expressly disclaims being a specialist in the field of plant geography. CAMPBELL[4] has issued a volume which should be in the hands of all intelligent world travelers, for one without technical knowledge will have no difficulty in following through the volume. At the same time the student of botany desiring to get a picture of the large features of world vegetation will find this book very helpful. The author is himself a great world traveler who has visited many countries in search of morphological material, and he has also published various important phytogeographic papers on Hawaii and other lands.

The introduction deals with the succession of plants in geologic time, then follows a chapter on climatic zones. Seven chapters deal with the vegetation of the different lands as follows: Eurasia and North Africa, Atlantic and Central United States and Canada, the Rocky Mountains and Pacific slope, Africa and Continental Asia, Malaya and Polynesia, the Neotropical Regions, and the South Temperate Zone. The volume is profusely and well illustrated by photographs taken by the author and others.—H. C. COWLES.

Soil microbiology

Once in a while a book appears that one knows is destined to become a classic in its particular field, and to pass through edition after edition. Such a book is WAKSMAN'S[5] new work, an encyclopedic summation of our knowledge thus far in the literature of the little things that live in the soil unseen and for the most part unthought of, yet vastly influencing our lives for good or ill.

Waksman chooses his text well, from PASTEUR: " . . *le rôle des infiniments*

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[4] CAMPBELL, D. H., An outline of plant geography. 8vo. pp. ix+392. *pls. 52. figs. 101.* New York: Macmillan Co. 1926.

[5] WAKSMAN, SELMAN A., Principles of soil microbiology. 8vo. pp. xxviii+897. *pls. XIX. figs. 77.* Baltimore: Williams & Wilkins. 1927. $10.00.

petits m'apparaissait infiniment grand . . ," and the thoroughness with which he undertakes his task measures well with his appreciation of its importance. Bacteria, protozoa, myxomycetes, fungi, algae, worms, all the swarming invisible life of the earth: he neglects none of them. Nor does he neglect an avenue of approach that promises to lead into a region whose exploitation will be profitable: taxonomy, physiology, ecology, physics, chemistry, each comes in for its share of attention, with special chapters on oxygen, nitrogen, and sulphur relations, and whole series of chapters on the interactions between the living and nonliving organic masses in the soil. He pins his generalizations down with tables of quantitative data, 94 of them. He has impounded and digested a huge literature; his classified list of useful reference books fills 8 pages, his index of authors 21, and his separate citations reach the impressive total of 2543. In quantity the book is impressive; in quality it is excellent.—F. THONE.

Palladin's Plant physiology

The PALLADIN-LIVINGSTON[6] textbook of plant physiology has appeared in its third English edition. Only a few changes have been made from the second edition. The list of books for reference preceding the table of contents has been extended considerably, with a gain in value to the general student, as many of the references added are of the newer publications. Some of the footnotes have been revised to include recent publications also, and an account of the ASKENASY experiment has been inserted on pages 147–150.

One small error has persisted through all three editions, and attention is called to it, in the interests of students who may try repeating the demonstration of oxygen liberation during photosynthesis by use of the SCHÜTZENBERGER reagent. The directions are given on page 5, but sodium sulphite, Na_2SO_3, cannot be used for this purpose. The original formula calls for sodium hyposulphite. Unfortunately sodium thiosulphate, $Na_2S_2O_3$, has frequently been called sodium hyposulphite, and goes as "hypo" in photography. The proper chemical to use is the true sodium hyposulphite, $Na_2S_2O_4$.

The book has been very useful since its first appearance, and will no doubt continue to be used by students of plant physiology until something better has been produced in the way of a textbook.—C. A. SHULL.

NOTES FOR STUDENTS

Boron, a plant nutrient.—Since the first discovery of boron in plants 70 years ago, the widespread occurrence of boron in fruits, leaves, and other parts of plants throughout the plant kingdom has been abundantly demonstrated. The toxic action of excessive quantities of boric acid and borax has also been observed by many investigators. Occasional suggestions also were made of stimu-

[6] PALLADIN, V. I., Plant physiology, translated by LIVINGSTON, B. E., 3d ed. 8vo. pp. xxxvi+360. Philadelphia: Blakiston. 1926.

lating action of very dilute solutions of boron compounds; but in recent years evidence of a more important relationship of boron to plant life and plant growth has been obtained.

Since the work of AGULHON, 17 years ago, experiments have been in progress at the Rothamsted Experiment Station which demonstrate the importance of boron to some of the legumes. The work was begun with *Vicia faba*, and Miss WARINGTON'S[7] results proved conclusively that boron was needed by the broad bean. Plants grown in nutrient solutions spectroscopically free from boron died in a characteristic manner, the growing points being the parts mainly affected. The amount of boron needed was so small that mere traces were sufficient to promote normal growth. The root systems without boron were short and stumpy, whereas they were long and well branched if traces of boron were present.

The abnormal behavior of this plant in the absence of boron is irrespective of the pH of the nutrient solution, of the aeration, of the nature of the substratum, or of the presence or absence of nodules on the roots. The relation of boron to the nodule development on *V. faba* was investigated by BRENCHLEY and THORNTON,[8] who claim that boron is indispensable to the legume bacteria which use it in some way in connection with the nitrogen fixation process, the synthesis of organic nitrogen compounds from atmospheric nitrogen.

The various anatomical changes in the structure of *V. faba* by the absence of boron were reported by Miss WARINGTON,[9] who found that both stem and root structures are modified by lack of boron. Cambium cells become hypertrophied, followed by degeneration, or they may disintegrate without hypertrophy. The phloem and ground parenchyma also show some disintegration, and the xylem is poorly developed. Whether these effects are due to the absence of some direct action of boron, or to indirect consequences, is not yet certain. It is suggested that the various changes are correlated with the meristematic activity of the tips of stems and roots.

Although any boron-containing compound, even the insoluble borates, supply the plants sufficiently, no other element can be substituted for boron in this connection. BRENCHLEY and WARINGTON[10] have tested 52 other elements,

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[7] WARINGTON, KATHERINE, The effect of boric acid and borax on the broad bean and certain other plants. Ann. Botany **37**: 629–672. 1923.

[8] BRENCHLEY, W. E., and THORNTON, H. G., The relation between the development, structure, and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. Proc. Roy. Soc. London B. **98**: 373–398. 1925.

[9] WARINGTON, KATHERINE, The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Botany **40**: 27–42. 1926.

[10] BRENCHLEY, W. E., and WARINGTON, KATHERINE, The rôle of boron in the growth of plants. Ann. Botany **41**: 167–187. 1927.

with special emphasis on manganese without finding a successful substitute. They have extended their observations to include several leguminous plants, melon, cereals, and candytuft. The legumes and melon require boron, but cereals and candytuft complete their development without it. In this their results do not agree entirely with those of SOMMER and LIPMAN,[11] who found that boron was essential to the complete development of barley, sunflower, cotton, buckwheat, castor bean, flax, and mustard. BRENCHLEY and WARINGTON think the distinction between those plants that need boron and those that do not may not necessarily be qualitative, but rather quantitative. Some plants may have enough boron in their seeds to supply the amount needed for complete development.

Renewal of the nutrient solution by means of drip cultures in which renewal was complete every 24 hours retarded the appearance of boron starvation for from 3 to 6 weeks, but did not prevent ultimate death of the plants. In their most recent paper BRENCHLEY and WARINGTON express the belief that there is an association between boron and the absorption and utilization of calcium. The boron is not considered an ordinary catalyst, but seems to be absorbed and combined, or removed from action in some way, so that a constant supply seems to be needed.

The amount of boron needed by plants is so small, about one-half part per million, that it can never be a problem in nature. All soils naturally contain enough boron that to demonstrate the need of plants for this element requires unusual precautions in the way of insoluble glass vessels, recrystallized salts, and protection from dust particles. Some of the other elements now considered nonessential for plant growth will no doubt be found necessary when we actually provide the plant with an environment entirely free of the elements in question. Plants have probably never been grown in the complete absence of silicon, for instance, nor of chlorine. Until they are, we cannot decide whether these elements are necessary or not to the normal growth of plants. Boron has now been shown to be indispensable in minute amounts, for the proper development of many of the higher plants.—C. A. SHULL.

North American Flora.—The fourth part of volume 34 contains the presentation of 3 tribes of Carduaceae by AXEL RYDBERG. The Liabeae include 5 genera, 3 of which are new, namely *Sinclairiopsis*, *Megaliabum*, and *Liabellum*. The largest genus is *Sinclairia*, with 18 species. Nearly all of these forms have been segregated from *Liabum*. The tribe Neurolaeneae contains 2 genera, with 15 species, 5 of which are new. The tribe Senecioneae is presented with 15 of its 27 genera. Much the largest genus is *Arnica*, with 107 species, 31 of which are new. The 14 remaining genera include 33 species, 5 of which are new. Among them are the following new genera: *Raillardiopsis*, *Pseudobartlettia*, and *Psathyrotopsis*.—J. M. C.

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[11] SOMMER, A. L., and LIPMAN, C. B., Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1:231–249. 1926.

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